UNVERSITÉ DE SHERBROOKE

STUDIES ON THE RENAL EFFECTS OF BRADYKININ IN ANESTHETIZED DOGS: function and regional extravasation of albumin.

par

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ABBREVIATIONS

ACE	Angiotensin converting enzyme
ADH	Antidiuretic hormone ,Vasopressin
BK	Bradykinin
C_{inulin}	Clearance of inulin
C_{PAH}	Clearance of PAH
CPM	Counts per minute
CTX	Cortex
EB	Evans Blue dye
EDRF	Endothelium derived relaxing factor
GFR	Glomerular filtration rate
I-R	Ischemia reperfusion
IM	Inner medulla
OM	Outer medulla
PAH	Para aminohippurate
PAP	Papilla
PGE_2	Prostaglandin (E_2)
RBC	Red blood cell
RPF	Renal plasma flow
$U_{Na}V$	Sodium excretion rate
UV	Urine flow rate

RESUME

Le rein est l'organe principal impliqué dans la régulation des composantes et du volume liquidien du corps. L'anatomie particulière et les propriétés fonctionelles des éléments vasculaires et tubulaires du rein rendent possible l'excrétion et la rétention sélective de solutes et d'eau. Les mécanismes impliqués dans la régulation de ces fonctions ne sont pas entièrement connus mais impliquent en grande partie l'action d'hormones agissant sur des sites intrarenaux particuliers. Nous avons choisi d'étudier l'action rénale de la bradykinine parce que celle-ci est produite normalement au niveau du rein et elle semble être impliquée dans certaines pathophysiologies (e.g. l'hypertension ou l'inflammation). De plus, l'action de la bradykinine est modulée par diverses pharmacothérapies.

Dans un premier temps nous avons étudié l'action de faibles doses de bradykinine infusées dans l'artère renale du chien, en condition de diurèse aqueuse ou lors d'une charge élevée d'urée afin de mieux comprendre l'action de la bradykinine sur les segments distaux du nephron. Nous observont que la diurese et la natriurese induite normalement par la bradykinine persiste toujour et que l'excretion d'urée est inchangée dans ces deux condition experimentale. Ceci nous indique que l'action tubulaire de la bradykinine n'est pas exclusivement du a la modulation des effets de la vasopressine

Nous décrivons ensuite le développement d'une technique permettant l'évaluation de l'extravasation d'albumine et le contenu en eau de différentes regions du rein. Ce phénomène vasculaire a reçu peu d'attention quant à ces effets sur la fonction rénale. Nos résultats valident l'utilisation du Bleu Evans comme marqueur d'albumine dans les conditions physiques particulaire au rein de pH réduit et d'osmolaritée élevée. De plus nous demontrons que le Bleu Evans mesuré se retrouve surtout dans le compartiment extravasculaire.

Nous avons utilisé cette technique dans le rein pour localiser des sites d'action vasculaire de la bradykinine. De plus, nous avons caractérisé le rôle des sous-types de récepteurs aux kinines a l'aide d'antagonistes selectifs. Nous démontrons que la bradykinine infusées dans le rein augmente l'extravasation d'albumine au niveax du cortex seulement. Deux inhibiteurs de l'enzyme de conversion de l'angiotensine ont également été infusé dans le rein afin d'élever la concentration de bradykinine endogène. Finalement, puisque l'ischemie-reperfusion rénale est une situation pathophysiologique courante impliquant des phénomènes d'inflammation locale dans le rein, nous nous sommes intéressés à decrire le profil d'extravasation de l'albumine dans de tèlles conditions afin.d'établir l'implication possible des kinines. Nos résultats démontrent un profil d'extravasation d'albumine different de celuis produit par la bradykinine.

Les résultats présentés dans cette études apportent des connaissances nouvelles sur l'action de la bradykinine au niveau du rein et également sur les mechanisme de régulation de la fonction du rein.

SUMMARY

The kidney is a primary regulator of body fluid composition and volume. The particular architecture and functional properties of the tubular and vascular elements of the kidney and their interaction allows for the selective excretion or retention of various solutes and even of water itself. The mechanisms leading to certain functional responses are extremely complex and not yet fully understood but are in large part regulated by hormones acting at specific locations in the kidney. Of the many hormones involved we chose to study bradykinin because it is normally produced in the kidney, it may be involved in pathophysiological processes (eg. hypertension and inflammation) and is certainly affected by common pharmacotherapeutic interventions.

We first present studies in which dogs, infused with either hypotonic saline or an isotonic solution of urea, received a small dose of bradykinin via the renal artery. The demonstration of dissociable diuretic and natriuretic effects in these conditions supports the idea that bradykinin acts in the distal segments of the nephron.

We then describe the development of a technique allowing us to determine regional changes in the extravasation of albumin from the renal microcirculation as well as changes in tissue water content. We present studies validating the use of Evans Blue dye as a marker for albumin and experiments that validate the assumption that the dye we measure is indeed extravascular.We show that the distribution between renal zones is very heterogeneous and changes under various conditions of altered renal function.

We applied the technique to identify renal vascular sites of action of bradykinin. Furthermore, the role of receptor subtypes in this response was determined using selective receptor antagonists. Intrarenal bradykinin infusion is shown to exclusively change cortical albumin extravasation. We also demonstrate completely different profiles of two different angiotensin converting enzyme inhibitors (products known to reduce kinin degradation) on function and albumin extravasation. Finally, the effects on albumin extravasation was determined in dogs subjected to renal ischemia reperfusion injury since this common pathophysiological condition is known to involve a localized inflammatory reaction (perhaps involving bradykinin). This did not appear to be the case.

We submit this work in the hope that it may provide insight into the renal actions of bradykinin as well as on the mechanisms regulating renal function in general.

INTRODUCTION

The kidney is a primary regulator of body fluid composition and volume. The particular architecture and functional properties of the tubular and vascular elements of the kidney and their interaction allows for the selective excretion or retention of various solutes and even of water itself. The mechanisms leading to certain functional responses are extremely complex and not yet fully understood but are in large part regulated by hormones acting at specific locations in the kidney. Of the many hormones involved we chose to study bradykinin because it is normally produced in the kidney, it may be involved in pathophysiological processes and is certainly affected by common pharmacotherapeutic interventions. We hope that the results of our study will not only provide insight on the renal actions of bradykinin but also on the mechanisms of renal function in general.

1.1 KININS

A group of physiologically active peptide hormones, the kinins, are liberated following their cleavage from circulating or localized precursors (kininogens) by a group of serine proteases called kallikreins, also found in the vascular or interstitial compartment. The most studied biologically active kinin is the nonapeptide bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg). Kinins may be degraded into inactive fragments by various peptidases or into biologically active fragments (des-Arg^9 kinins) by carboxypeptidases that cleave the C terminal arginine. Bradykinin (BK) has a wide range of activities

including those observed in inflammatory reactions such as nociception and capillary permeabilization. Kinins are also involved in the regulation of vascular tone and is best known for its vasodilatory action (REGOLI, 1987; REGOLI & BARABE, 1980), however the response is not uniform and in some in vitro preparations vasoconstriction occurs (TSURU *et al.,* 1974). As we shall see, in certain tissues, BK is known to mediate the transport of ions such as Na^+, Cl^- and HCO_3^-. Kinins circulate in plasma but are also suspected of playing an important autocrine role in physiology and pathophysiology. Certain organs such as the lung and kidney are known to produce, degrade and respond to these hormones.

1.1.1 Receptor subtypes

A number of factors could explain the wide range and heterogeneity of responses elicited by kinins. First of all, two receptor types have been described following the observation that opposite orders of activity were elicited by different kinins on various vascular preparations (REGOLI, 1987; REGOLI & BARABE, 1980). The B_1 receptor is selectively responsive to kinins that have lost the terminal arginine group (des-Arg^9 Kinins) while B_2 receptors are preferentially acttivated by kinins posessing the terminal arginine. Unfortunately there is no clear relation to be found between receptor type a second messengers or response elicited.

The distribution of kinin B_1 receptors is less extensive than that of the B_2 type. The former is found predominantly in vascular beds and often co-located with the latter type. There are reports of B_1 receptors

on bone cells of embryonic mice (LJUNGGREN and LERNER, 1990) as well as rabbit (MARCEAU and TREMBLAY, 1986) and human (GOLDSTEIN and WALL, 1984) fibroblasts. On the other hand, B2 receptors have been identified in practically every tissue. It is worth noting that there is good evidence that the expression of either receptor subtype can be induced; B_1 type by tissue injury (see MARCEAU *et al.*, 1983) or mediators of immune responses (De BLOIS *et al.*, 1991), B_2 type by the action of oncogenes (RUGGIERO *et al.*, 1989), steroid hormones (ROSHER *et al.*, 1990) and IL-1 (BATHON *et al.*, 1992). Changes in cell surface receptor expression may result from changes in *de novo* synthesis (REGOLI *et al.*, 1978) or ligand induced internalization (WOLSING and ROSENBAUM, 1991).

1.1.2 Receptor-effector coupling

Depending on the tissue type, kinin effects are known to involve a wide range of second messenger mechanisms. Thus, the receptor effector coupling of B_1 type receptors in rabbit isolated aorta is dependent on external Ca^{++} (CALIXTO and MADEIROS, 1992a) but does not appear to require protein kinase C activation (CALIXTO and MADEIROS, 1992a) or prostaglandin release (REGOLI and BARABE, 1980). Relaxation mediated by this receptor is endothelium independent in the rabbit mesenteric artery (CHERRY *et al.*, 1982) in contrast to that in the canine mesenteric vein where endothelium derived PGI_2 is the mediator (TODA *et al.*, 1987). Cell culture studies have shown that B_1 receptor agonists can cause the release of

EDRF/NO in bovine aortic and pulmonary artery endothelial cells (D'ORLEANS-JUSTE *et al.*, 1989; SUNG *et al.*, 1988)). This is particularly interesting since the natural production of B_1 receptor ligands by kininase I results in the release of arginine, a precursor of EDRF (LEAF *et al.*, 1989). Finally, B_1 receptors are involved in the modulation of DNA synthesis, via activation of protein kinase C in rat mesangial cells (ISSANDOU and DARBON, 1991)

The stimulation of B_2 receptors has been associated with the activation of most second messenger systems, depending on the tissue. The most consistent finding is that B_2 receptors mediate the formation of IP_3 and diacylglycerol by activating phospholipase C, leading to Ca^{++} release and protein phosphorylation (FARMER and BURCH, 1992). Another common response mediated by B_2 receptors is the release of arachidonic acid metabolites via the activation of phospholipase A_2 (BURCH and AXELROD, 1987; SLIVKA and INSEL, 1988). There is a notable absence of reports showing BK mediated activation of adenylate cyclase, however there is a report of cAMP generation in the rat isolated duodenum (LIEBMANN *et al.*, 1987). In fact BK mediated increases in cAMP is generally believed to result from prostaglandins (BURCH, 1990; DIXON *et al.*, 1990). In porcine aortic endothelial cells, BK is known to increase EDRF and PGE_2 (GRYGLEWSKI *et al.*, 1986) as well as cGMP (BOULANGER *et al.*, 1990). There is clear evidence that all the effects mentioned above involve the activation of specific G proteins (GRAIR *et al.*, 1992; BURCH and AXELROD, 1987; SLIVKA and INSEL, 1988). There is also evidence that more than one of these pathways can be activated within the same cell (BURCH and AXELROD, 1987; KAYA *et al.*,

1989) although it is likely that different G proteins are involved (CHUANG and DILLON-CARTER, 1988).

1.1.3 Ion transport

The regulation of ion transport by kinins has been measured *in vitro* in a variety of transport epithelia. There have been numerous reports detailing the effects of bradykinin on electrogenic Cl- transport. More recently, it has been shown that the transport of other anions such as HCO_3^- and cations such as Na+ can be regulated by kinins. However, the receptor types and the second messengers involved may differ between tissues (even when the transport of the same ion is considered). Furthermore, there is variability in the disposition of receptors on different epithelia, that is they may be found on one or both sides of the epithelium. As with other effects mediated by BK, there is evidence that the generation of eicosanoids plays a part in the effects on ion transport. Also, the actions of kinins are often Ca++ dependent.

The direct effect of bradykinin on Cl- transport was first described in the rat colon (CUTHBERT and MARGOLIUS, 1981). In the guinea-pig ileum, B_2 receptors located on the mucosal side mediate Cl^- secretion (GANGINELLA and KACHUR, 1989). In both this preparation (MUSCH *et al.*, 1983) and in rabbit ileum (HOJVAL *et al.*, 1983) the release of prostaglandins by BK plays a part in its effects. Similar results are obtained from the mucosal layer of the rat colon (BARON *et al.*, 1987; PERKINS *et al.*, 1988; PHILIPS and HOULT, 1988). There is also a report of inducible B_1 mediated Cl^- transport in

the rat colon following *in vivo* injury with acetic acid (KACHUR *et al.*, 1986).

Ion transport in other tissue types also responds to bradykinin. The secretion of Cl^- from isolated mucosal epithelial cells of the canine lung is increased by BK and accompanied by a release of prostaglandins (LEIKAUF *et al.*, 1985). This also appears to be a B_2 mediated effect (RANGACHARI *et al.*, 1990). BK applied to the mucosal side of the guinea-pig gallbladder was shown to increase bicarbonate secretion via a prostaglandin mediated mechanism (BAIRD and MARGOLIUS, 1989). Increased Na+ absorption and Cl- secretion in nasal epithelial cells is induced by a BK mediated increase in basolateral K+ conductance (CLARKE *et al.*, 1992). In vascular endothelial cells BK is known to mediate the activity of the Na+/K+/ 2 Cl- co-transport system that is responsible for cell volume regulation (O'DONNEL, 1993).

Finally, as will be discussed later, kinins and kinin receptors are found on and around the collecting duct in the kidney and BK is known to directly affect Na+ and Cl- transport there. In addition there is evidence that BK modulates the response of this tissue to other hormones such as ADH.

1.1.4 Vascular reactivity

Kinins can have an effect on three distinct components of the vascular system; arteries, veins and capillaries (the latter to be discussed with respect to permeability in the following section). The arteries are primarily resistance vessels that play a role in regulating

the distribution of blood flow to the different organs and vascular beds. Regulation of vascular resistance in these vessels is closely linked to changes in blood pressure and cardiac afterload. It is generally agreed that systemic infusion of kinins decreases arterial blood pressure by reducing arteriolar resistance (GREISBACHER *et al.*, 1989; LEMBECK et al, 1991). The regional effects of arterial dilatation and the direct effects of BK on capillaries and veins are heterogeneous and not yet clearly defined. The regulation of venous tone can affect cardiovascular parameters in different ways. At the microvascular level, the relative resistance to flow through a capillary network imposed by the veins determines the intracapillary hydrostatic pressure, and consequently plasma extravasation. At the systemic level, the venous system serves as a reservoir or pool for blood allowing for the maintenance of arterial blood pressure when arterial tone changes. Finally, venous tone dictates the preload pressure of blood delivered to the heart and thus an effect on cardiac efficiency.

Of primary importance in the vascular action of BK is the endothelial cell. The microvascular endothelium serves as a blood compatible barrier regulating the passage of blood borne substance with extravascular components. In both the microcirculation and larger vessels this tissue also serves as an important transducer between circulating hormones and/or vascular smooth muscle. With respect to kinins, endothelial cells are clearly shown to elicit the production of eicosanoids such as PGE_2 or PGI_2 and the synthesis of EDRF. However, the relative contribution of these two mediators varies from one tissue to another and between species.

Studies have shown that kinins receptors of both types are also found on arterial and venous smooth muscle. This and the fact that inducible B_1 receptor types could be observed on denuded vessel preparations suggests a role for kinins in pathophysiological responses. Alternately, under normal conditions, these receptors may respond to kinins that have passed the endothelial barrier or to locally generated kinins. With respect to the latter proposal, kallikrein (which converts kininogens to active kinins) was reported to induce vasorelaxation via a B_2 receptor mediated mechanism in canine coronary arteries, suggesting the presence of endogenous kininogens in vascular tissue (MOMBOULI and VANHOUTTE, 1992).

The distribution of receptor subtypes on vascular smooth muscle and endothelium vary widely between vessel types and the species studied as do the responses they elicit. This heterogeneity between vessels and species has been extensively reviewed (REGOLI and BARABE, 1980). For example, in the dog, common carotid artery tone is reduced by BK (D'ORLEANS-JUSTE *et al.,* 1985; REGOLI *et al.,* 1986) but the internal and external carotid contract (TODA, 1987). The aorta of most species contracts in response to BK but not in the rat (BARABE, 1976). In canine isolated blood vessels, bradykinin acting via B2 receptors relaxes $PGF_{2\alpha}$ precontracted coronary arteries (TODA *et al.*, 1987), but in the guinea-pig mesenteric and rabbit jugular vein, B_2 receptors mediate a vasoconstrictor response (GAUDREAU *et al.,* 1981a, b). B_1 receptors mediate contraction of the rabbit aorta (RHALEB, 1990) and celiac artery (RITTER *et al.,* 1989). In contrast, B_1 antagonists prevent prostaglandin mediated relaxation of the mesenteric vein in the dog (TODA *et al.,* 1987).

Renal vascular localization of kinin receptors has been demonstrated but intrarenal distribution and receptor types have yet to be clearly defined. In the rat, both receptor subtypes are found in the renal vasculature (GUIMARAES *et al.*, 1986). The vasodilator action of kinins in renal artery preparations is well documented in both untreated and precontracted tissue (REGOLI and BARABE, 1980; TODA, 1987). This effect is endothelium dependent since canine renal arteries denuded of their endothelium contract via B1 receptor action. (REGOLI *et al.*, 1990; RHALEB *et al.*, 1989). Cell cultures derived from renal cortical arterioles were shown to produce PGE2 in response to BK (DUSSAULE *et al.*, 1989). It has been shown in isolated canine renal veins that BK elicits contraction (TSURU *et al.*, 1974) but at doses three orders greater than those used in other venous preparations. Finally, recent reports show that both kinin receptor subtypes are found in the glomerular mesangium (Bascand *et al.*, 1993). This is curious since acute infusion of bradykinin is not known to affect glomerular filtration rate.

1.1.5 Vascular permeability

Both receptor types are known to be involved in the events causing and amplifying inflammatory reactions to injury and disease (see REGOLI and BARABE, 1980). Included in these events is an increase in the extravasation of plasma and circulating proteins into the interstitium (SARIA *et al.*, 1983), leading to the formation of edema. BK can increase extravasation by altering pre- and post-capillary vascular tone (and thus local hydrostatic pressures) and

perhaps more importantly by provoking cellular disjunction of vascular endothelium (KEAHEY *et al.*, 1991; SVENSJO *et al.*, 1979). In the hamster cheek pouch the latter mechanism was recently shown to be mediated by B_2 type receptors and dependent on protein kinase C (MURRAY *et al.*, 1991).

In accordance with these results, others (LEMBECK *et al.*, 1991; PLANTE *et al.*, 1993; SAKAMOTO *et al.*, 1992) have shown that this same receptor subtype accounts in large part for increased permeability in the skin and various organs of the rat following systemic BK infusion. Those studies described the heterogeneous permeability characteristics of different vascular beds and the changes in albumin extravasation induced by BK. In particular, the capillaries of the lung, kidney and duodenum were preferential sites of BK action in which an increase in albumin extravasation occurred. These effects in the lung and kidney were completely abolished by pretreating the animals with a selective B_2 receptor antagonist DArg^0-Hyp^3-DPhe^7-bradykinin, while only partial return to baseline values in the duodenum obtained. It is interesting to note that although BK alone did not significantly change capillary permeability characteristics in the skeletal muscle a significant decrease in albumin extravasation was seen in this tissue when animals were pretreated with the B_2 receptor antagonist. This could be indicative of a physiological role of endogenous kinins in the rat skeletal muscle.

Bradykinin induced changes in hydrostatic conductivity were localized in the arteriolar and true capillaries of frog mesentery, while venular water flux was unchanged (WILLIAMS and HUXLEY, 1993). BK is thought to be released and to cause plasma extravasation in

response to carrageenin (WIRTH *et al.*, 1991), urate crystals (DAMAS and REMACLE-VOLON, 1992), caerulein (GRIESBACHER and LEMBECK, 1992) and phospholipase A_2 (CIRINO *et al.*, 1991) since B_2 receptor antagonists inhibit the response

The regulation of vascular permeability by the kallikrein kinin system may be involved in the development of certain pathologies. It was reported that in young SHR rats a B_2 receptor antagonist was able to reverse characteristic changes in albumin extravasation in different vascular beds, before the onset of hypertension, suggesting a role for BK in the onset of their disease (PLANTE *et al.*, 1992).

1.1.6 Renal localization (non vascular)

A role in renal function may be inferred from studies that have localized the intrarenal distribution of the elements in the kallikrein kinin system (KKS). A high concentration of ACE/kininase II is found on the luminal surface of the proximal tubule (REGOLI and BARABE, 1980; SHARMA, 1988; GUDER and HALLBACH, 1988). Since this enzyme completely inactivates kinins (REGOLI and BARABE, 1980; SHARMA, 1988; GUDER and HALLBACH, 1988), we can assume that no active filtered kinins reach the loop of Henle.

The production and receptor sites of kinins have been localized in and around the distal tubule and collecting duct (GUDER and HALLBACH, 1988). Kallikrein has been localized in the early distal tubule. In the late distal and cortical collecting segments a secretion of kinin precursor, kininogen, is observed. Furthermore, these segments are known to establish a close anatomical relationship with the

afferent arterioles in the region of the juxtaglomerular apparatus (VIO *et al.,* 1992). This could allow for a BK mediated signaling mechanism between the two structures. Receptor sites and inactivating enzymes are located in and around the medullary collecting tubule on both basal and luminal sides (GUDER and HALLBACH, 1988). Furthermore, interstitial cells of the matrix between tubule and vascular elements of the medulla also have receptors (FREDERICK *et al.,* 1985; GUDER and HALLBACH, 1988). This localization further suggests a potential interaction between the different elements of the kidney.

1.1.7 Renal physiological effects

Bradykinin has long been known to induce diuresis, natriuresis and increases renal blood flow (RPF) with little or no change in glomerular filtration rate (GFR) (DEFELICE and BROUSSEAU, 1988; FLAMENBAUM *et al.,* 1979; GRANGER and HALL, 1985; STEIN *et al.,* 1972; YUN *et al.,* 1982). However, the mechanisms of these actions have not yet been entirely established. Concurrent increases in renal blood flow, diuresis and natriuresis have led to the frequent suggestion that the effects of BK infusion may be the result of its vasodilator action and/or blood redistribution to the medulla (EARLEY and FREIDLER, 1966; FADEM *et al.,* 1982; FURTADO 1981; WEBSTER and GILMORE, 1964; WILLIS *et al.,* 1969).

Alternately, BK infused into the renal artery may directly affect tubular function. This idea is supported by the fact that all the elements of the kallikrein/kinin system have been localized in and

around renal tubules (FREDERICK *et al.,* 1985; GUDER and HALLBACH, 1988; HELLBERG and KALLSKOG, 1988; TOMITA and PISANO, 1984) and that kinins have been shown to inhibit ADH activity *in vitro*, by interfering with post receptor transduction mechanisms (CARVOUNIS *et al.,* 1981; DIXON *et al.,*1989; FURTADO 1981; SMITH *et al.,* 1989). In rabbit inner medullary collecting duct cells, BK was shown to inhibit conductive Na+ entry on the luminal side (ZEIDEL *et al.,* 1990), suggesting that natriuresis could result from direct tubular action. This may involve phospholipase C activation since this was shown to increase in similar preparations (DIXON *et al.,*1989; WEISS and INSEL, 1990) but not in rat collecting ducts (ROUCH *et al.,* 1991).

We have previously demonstrated, using a small dose of BK and selective receptor antagonists infused directly into the left renal artery, that significant diuresis and natriuresis could be obtained in the absence of concurrent changes in GFR or RPF (LORTIE and PLANTE, 1990; LORTIE *et al.,* 1992). In those studies, we noted a dissociation of the diuretic and natriuretic effects of BK, diuresis being regulated by B_2 receptors and natriuresis by the B_1 type. Interpretation of this functional data placed potential tubular receptor sites beyond the thin ascending limb of Henle. This was in accord with evidence demonstrating the inhibition of ADH mediated water permeability (CARVOUNIS *et al.,* 1981; FURTADO 1981; SMITH *et al.,* 1989) known to occur in the collecting ducts. Similar interaction with other ADH regulated events such as sodium transport in the cortical collecting duct or urea transport in the papillary collecting duct remain unknown. The interpretation of our functional data was based

on the fact that global RPF and GFR was unchanged by the small dose of BK we used and that its effects were rapidly reversible. However, the possibility of regional changes in renal vascular hemodynamics could not be excluded in these studies.

We therefore decided to test the hypothesis that BK antagonized ADH mediated tubular effects by experimenting on animals undergoing water diuresis. This condition, known to inhibit ADH release and minimize free water reabsorption (CHOU *et al.*, 1990), could help to identify the means whereby BK causes its natriuretic effects. Also, this condition could minimize any vascular effect of BK in the medulla, since blood flow there would already be increased (CHOU *et al.*, 1990). We also decided to investigate the effects of BK in dogs having received a urea load to induce an osmotic diuresis. In these conditions the obligatory loss of free water stimulates the release of ADH (BANKIR *et al.*, 1987; YANCEY *et al.*, 1989) and could accentuate any mediator effects by BK. Also, this condition may enhance urea transport and amplify any effect that BK might have on this particular function. This idea developed from the fact that different carriers are responsible for both water and urea transport in the terminal segments of the collecting duct but that both are mediated by ADH (KNEPPER and STAR, 1990). Thus, if BK had an inhibitory effect on ADH mediated free water reabsorption it might also affect urea transport. In previous experiments on normal animals we had been unable to demonstrate such an effect, but we determined to test if this might be due to low transport rates.

The physiologically opposing effects of ADH and BK on blood flow in the medullary microcirculation raises questions as to their

potential interaction there. Also, since BK is known to be a mediator of vascular permeability and inflammation in other capillary beds, one of its renal vascular properties may be to alter the osmotic and oncotic gradients established in different regions of the kidney, a similar role for ADH has not been studied. This could be expected to have a major effect on function and/or concentrating ability but would not be detected using conventional methods of measuring renal hemodynamics.

1.2 THE REGULATION OF FLUID AND SOLUTE FLUX IN THE KIDNEY: TRANSPORT, DIFFUSION AND CONVECTION ACROSS VASCULAR AND TUBULAR TISSUE.

From the cell membrane of the basic unicellular organism to the complex architectural structure of the nephron and vasa recta in the medulla of the mammalian kidney, the regulation and maintenance of physical and chemical gradients across semipermeable cell membranes and epithelia dictates the movement of water and solutes. This movement towards equilibrium is directed by concentration gradients, as described by Donnan, and the net pressure gradient established by differences in osmotic, hydrostatic and oncotic forces , as described by Starling. In mammalian kidneys, it is the particular spatial arrangement of nephron segments and renal blood vessels which confers the ability to establish a hyperosmotic environment in the medulla and thereby regulate the tonicity of urine, while transport regulation across nephron epithelia dictates its composition. The many

mechanisms involved in regulating urine volume and composition, so as to maintain body fluid homeostasis, are under the complex regulation of circulating and local mediators.

Because of the close functional association between the vascular and tubular elements of the kidney, it is perhaps not surprising to find that both can be sites of action for a common mediator. The location and characterization of receptors on nephron segments can be tested both *in vitro* and *in vivo*, providing manageable information that can help to understand functional responses. However, there is no clear understanding of the mechanisms whereby renal function is altered by the action of hormones on the renal microcirculation. This is due to the regional heterogeneity of the renal vascular system and our inability to accurately measure local vascular hemodynamic parameters *in situ* or to reduce the system into simple components that can be studied independently. Global renal hemodynamic parameters like renal perfusion pressure, renal plasma flow and glomerular filtration rate (RPP, RPF and GFR, respectively) are highly informative with respect to events that occur in the renal cortex where most of the renal blood circulates. However these measurements can tell us little about vascular events beyond the efferent arteriole or about regional flow distribution. The importance in understanding the renal effects of vasoactive hormones becomes critical when one considers the implication of these mediators in various pathophysiological processes, particularly hypertension and inflammation, and that they are a primary target of pharmacological intervention.

1.2.1 Intrarenal blood flow

Various parameters pertaining to the renal vascular system have been studied under different conditions. The role of renal blood flow redistribution to the medulla has attracted much attention with respect to its effect on medullary osmotic gradients. Many techniques have been developed in an attempt to determine flow rates in the medulla and extensive reviews have been published (CUPPLES, 1985; PALLONE *et al.,* 1990; PINTER, 1969). These methods aim to quantify either the local transit time of a vascular marker at the papilla tip, the initial accumulation rate of a vascular marker or the venous efflux kinetics of a "pulsed" marker. All have serious technical deficiencies, but provide remarkably reproducible results that can be used to demonstrate, at least qualitatively, changes in medullary flow. The role of hydrostatic pressure gradients on function has also received focused attention with respect to the regulation of glomerular filtration regulation by pre- and post-glomerular vascular tone. Also changes in medulla interstitial pressure as a result of increased local blood flow was investigated using implanted pressure transducers (GRANGER *et al.,* 1988; KRAIBI and KNOX, 1989). Finally, estimates of renal vascular volume have been calculated using silicone casts or the distribution volume of various vascular markers.

1.2.2 Albumin and the kidney.

It was the comparison of data obtained with the latter technique using tagged red blood cells and marked albumin which lead to the discovery of a large rapidly exchangeable pool of albumin in the renal medulla (LASSEN *et al.*, 1958; SOLTKOFF and LILLIENFIELD, 1967). Remarkably, although this phenomenon is well established, the literature has remained incredibly silent with respect to its implications. Since the greater number and size of ascending than descending vasa recta could not by itself account for the reabsorptive capacity of the kidney and that high albumin concentrations were observed in vessels at the papilla, the concept that high concentrations of intravascular albumin provides the driving force for the passage of free water from the interstitium to the ascending vasa recta was readily accepted and persists today (BANKIR and De ROUFFIGNAC, 1985; BANKIR *et al.*, 1987; KRIZ, 1982; PALLONE *et al.*, 1990).

Anatomical comparisons and recent studies of isolated perfused vasa recta leave no doubt that ascending vessels are far more permeable to albumin because they are fenestrated (KRIZ, 1982; PALLONE, 1992; SHIMAMURA and MORRISON, 1973). This implies that the gradient that was believed to occur across the ascending vasa recta membrane actually occurs between the interstitium and collecting duct and that a bulk flow of water with albumin allows water to leave the medulla. An important role of the oncotic forces generated by intravascular albumin in renal function has been well documented with regards to glomerular filtration and fluid reabsorption from proximal tubules by peritubular capillaries in the cortex (BRENNER *et al.*, 1969). It would appear then, that regional regulation of albumin

extravasation in the kidney may be an additional mechanism that contributes to the concentrating ability of the kidney. This is of particular interest to us because BK has endothelium permeabilizing properties. It is with this perspective that we decided to complement our ongoing studies into the renal functional effects of vasoactive mediators by studying regional changes in the extravasation of albumin under similar conditions.

1.2.3 Albumin extravasation studies in the canine kidney.

To do so we used systemically infused Evans blue dye (EB) since this has been shown to be a selective marker for serum albumin (LeVEEN and FISHMAN, 1947; RAWSON, 1942). Because the medulla of kidney presents a unique physical environment, pH may reach values in the range of 4.5-4.7 whereas urine osmolarity can exceed 2000 mOsm , we tested the binding characteristics of EB and albumin under different pH and osmotic conditions. To do so we took advantage of the fact that the maximal absorbance frequency of EB shifts from approximately 600 to 620 nm when it binds to albumin (RAWSON, 1942). As well, vascular volume studies were conducted using the ^{51}Cr distribution method (BEYER and GELARDEN, 1988; ROTHE *et al.*, 1979), enabling us to show that the EB we measured was indeed extravascular. The methods developed were used to document regional albumin extravasation during the first minute of marker infusion, in an effort to characterize differences in the rate of extravasation. Interpretation of data obtained following long perfusion times are confounded by heterogeneity in the size of the extravascular

albumin pool and its drainage by the lymphatic system in different tissues.

Along with albumin extravasation data we present the respective ratios of wet to dry tissue weight. In the studies we present, the dry weight of kidney tissue is assumed to remain constant since renal tissue is retrieved within 1 or 2 hours of experimentation onset. Thus changes in the ratio of wet to dry tissue weight are interpreted to reflect primarily changes in intra and/or extracellular fluid content. Note that under normal physiological conditions cell volume (i.e. cell water content) is maintained at very constant values by the active transport of small solutes.The question as to the relative contribution of vascular EB and fluid is resolved by the experiments where we measured vascular volume. As we will show, the wet to dry tissue weight ratios can be used to detect changes in tissue water content such as those causing edema. We present the results of albumin extravasation studies obtained in normal (control) animals and also in dogs infused with hypertonic saline or during water diuresis since the latter conditions would induce the production of highly dilute or concentrated urine, respectively.

After testing the model under normal conditions and during well studied conditions of salt and water imbalance, hypertonic saline infusion and water diuresis, we undertook the characterization of regional albumin extravasation following intrarenal infusion of BK. We also studied the effects of BK in combination with selective receptor antagonists since the latter were both shown by us to prevent BK induced increases in renal blood flow.

1.3 INHIBITORS OF THE ANGIOTENSIN CONVERTING ENZYME/KININASE II (ACE) IN RENAL FUNCTION.

Angiotensin converting enzyme/kininase II (ACE) is a nonspecific dipeptidylcarboxypeptidase that not only catalyses the conversion of angiotensin I to angiotensin II but also degrades vasoactive kinins (REGOLI & BARABE, 1980; SHARMA, 1988; McGIFF, 1980; FLAMEBAUM *et al.,* 1979). Inhibitors of this enzyme have enjoyed massive clinical success in the control of hypertension and the progression of certain renal diseases as seen in diabetics. In the past, much of their effects had been attributed to the reduction of circulating angiotensin II. The advent of selective and powerful angiotensin receptor antagonists have made it clear that significant effects of ACE inhibitors result from mechanisms other than angiotensin I conversion (COCKOFT, 1992; MITCHEL *et al.,* 1992). In this respect, the significant contribution of bradykinin to the blood pressure lowering effects of ACE inhibitors has been demonstrated by different groups under various conditions and was reviewed recently (GAVRAS, 1992).

Inter- and intraspecies isozymes of ACE have been reported. These can be located in circulating plasma, on the luminal side of endothelial cells and within the interstitial compartment (PEACH, 1977). The heterogeneous distribution of the enzyme(s), highly concentrated in the lung and the kidney (PEACH, 1977), is indicative of an important role in certain privileged vascular beds or tissues. In this respect, systemic Captopril infusion was shown to preferentially affects the microcirculation of these two organs (PLANTE *et al.,* 1993). In fact, this drug caused significant elevation in EB bound albumin

extravasation in the lung and kdney that could be prevented by pretreating animals with a selective B_2 receptor antagonist. This strongly suggested that Captopril infusion caused local increases in permeabilizing kinins that acted on B_2 receptors. In the kidney, much attention has been focused on the vascular and glomerular location of ACE in the autoregulation of GFR while the role of ACE found in the lumen of proximal tubules or the cortical interstitium has not been studied as extensively.

The initial success of Captopril has lead to the development of many analogues and some new classes of molecules able to inhibit ACE activity. The different pharmacokinetics of these substances can be expected to result in varying degrees of ACE inhibition in different regions throughout the system. Also, there is new evidence that some drugs in this class may have the particular property of changing endothelial cell production of EDRF (SALVEMINI *et al.*, 1993) Of the potent new substances developed, Perindopril is reported to have a high affinity for ACE and is more effective in blocking extravascular or tissue ACE (I.R.I.S, 1983; LOUIS *et al.*, 1993). Modulation of tissue ACE has generated great interest since its products and substrates (angiotensin I and bradykinin) are known to mediate effects other than vascular tone such as permeability (TONG *et al.*, 1992) and smooth muscle cell proliferation (BAUER *et al.*, 1990).

In studies performed in the anesthetized rat, Captopril and Perindopril (infused IV at 3.0 & 0.3 mg/kg, respectively) exerted different effects on glomerular filtration when renal perfusion pressure was reduced progressively within the range of autoregulation (down to 70 mm Hg) (PLANTE *et al.*, 1988). No change in GFR was observed

when Perindopril was infused while a loss of autoregulation during Captopril infusion caused a reduction of this parameter.The differences in effect of these two converting enzyme inhibitors are most likely related to local differences in sites of action on the renal vasculature since the doses used were shown to be equipotent in systemic blood pressure reduction (I.R.I.S ,1983)

It is clear that ACE inhibitors have an impact on two very important hormone systems in the kidney that are known to regulate tubular function, vascular reactivity and renal capillary permeability. The study of renal mechanisms affected by ACE inhibitors *in vivo* is complicated by its blood pressure lowering effects. In this respect, the intrarenal infusion of small quantities of drug, such that blood pressure is unchanged, is an ideal model for studying its direct renal effects. Also, since we had shown that Captopril and Perindopril elicited different renal functional responses in the rat we were interested in comparing the effects of these two drugs in the dog. We will show that characterization of the extravasation of albumin in different zones of the kidney helps to locate and differentiate sites affected by these drugs.

1.4 RENAL ISCHEMIA- REPERFUSION INJURY

Renal ischemia-reperfusion (I-R) injury is characterized by tubule swelling and vascular congestion in the outer medulla. There appears to be little consensus in the literature regarding the importance and time course of the different functional and structural disorders associated with renal ischemia and reperfusion. It is clear that I-R

initiates a series of events which alter renal function which can ultimately lead to irreversible renal failure (de ROUGEMONT *et al.*, 1982; MASON *et al.*, 1987; OLOF *et al.*, 1990; OLOF *et al.*, 1990; WOLGAST *et al.*, 1982). In experimental models, the severity of dysfunction seems to be dependent on a wide range of parameters including the duration of the ischemic period (HELLBERG and KALLSKOG, 1988; OLOF *et al.*, 1990; HELLBERG and KALLSKOG, 1988), the animals state of hydration (de ROUGEMONT *et al.*, 1982; JAMISON, 1973; MASON *et al.*, 1987; OLOF *et al.*, 1990; OLOF *et al.*, 1990; TORHORST *et al.*, 1982), the presence of a contralateral kidney (MASON *et al.*, 1987; OLOF *et al.*, 1990; YAGIL *et al.*, 1988) and the action of neutrophils (HELLBERG *et al.*, 1990; HELLBERG and KALLSKOG, 1988; KLAUSNER *et al.*, 1989; LINAS *et al.*, 1991; SHERIDAN *et al.*, 1991).

Histological findings indicate that there are certain key sites of tubular and vascular injury within the kidney following I-R injury that could lead to loss of renal function. Proximal tubule desquamation (de ROUGEMONT *et al.*, 1982; TORHORST *et al.*, 1982; WOLGAST *et al.*, 1982; YAGIL *et al.*, 1988), thick ascending limb swelling (MASON *et al.*, 1989; WOLGAST *et al.*, 1982), and erythrocyte accumulation in the outer medullary capillaries (de ROUGEMONT *et al.*, 1982; KARLBERG *et al.*, 1982; MASON *et al.*, 1987; OLOF *et al.*, 1990; VETTERLEIN *et al.*, 1986; WOLGAST *et al.*, 1982) are consistently reported in the early phase of reperfusion. However, the order in which these events occur, their relation to each other and the role they play in the cascade of events leading to renal failure is not yet clear.

The effect of I-R on intrarenal hemodynamics has been a primary focus of attention and it is clear that the outer medullary regions are particularly affected. Congestion and hypoperfusion after I-R is predominant in the microcirculation of the interbundle regions while blood flow in the intrabundle vasa rectae that supply the inner medulla and papilla appears to be rapidly restored (HELLBERG *et al.*, 1990; OLOF *et al.*, 1990; TORHORST *et al.*, 1982; VETTERLEIN *et al.*, 1986; YAGIL *et al.*, 1989). The congestion and swelling of the inner stripe capillaries is not believed to be hemostatic, since it is not prevented by heparin or salicylic acid (MASON *et al.*, 1987; WOLGAST *et al.*, 1982). Alteration of plasma hematocrit has provided surprising results since hemodilution confers significant protection while an increase in erythrocyte concentration results in a more rapid sludging of erythrocytes (HELLBERG *et al.*, 1990; MASON *et al.*, 1987; OLOF *et al.*, 1990; OLOF *et al.*, 1990). This supports the idea that a local hemoconcentration occurs in the inner stripe capillaries.

We present the results of clearance studies obtained before, during and after 30 min of unilateral renal artery occlusion. Furthermore, we present novel observations on the progression of intrarenal changes in albumin bound EB extravasation and tissue water content after 30 or 60 min of reperfusion.

1.5 SUMMARY

It is clear that kinins mediate a wide range of physiological and pathophysiological processes. Renal function is regulated to a large extent by the actions of hormones such as bradykinin and angiotensin

II acting on tubular, interstitial and vascular elements of the kidney. Dysfunction of these same hormonal systems is believed to be a major factor in the onset of essential and renal hypertension (eg. MARGOLIUS *et al.*, 1971; VIO *et al.*, 1992). Pharmacological agents have been developed, such as ACE inhibitors, which are extremely effective in lowering systemic blood pressure. Much of the renal effects of these drugs on renal hormone systems remains to be elucidated. One particular property of BK which has received little attention as far as renal function is concerned is its permeabilizing effect. Serum albumin gradients across capillary walls in the kidney have long been recognized as a key factor in the regulation of fluid reabsorption by peritubular capillaries in the cortex and free water reabsorption in the medulla. Thus some of the renal effects of BK may result from local changes in albumin extravasation. Capillary permeabilization is a hallmark of ischemia reperfusion injury in varous organs including the kidney. Since BK is known to be involved in inflammatory reactions we were interested in documenting the effects on vascular permeability of this pathophysiological condition.

Therefore, in an attempt to further our understanding of the renal effects of BK, we pursued previous studies of ours in which small doses of BK infused directly into the renal artery appeared to affect distal segments of the nephron. We studied the renal response to exogenous BK under conditions known to affect the function of distal segments of the nephron (i.e. water diuresis and urea loading). We also investigated the renal vascular effects of BK by developing a model allowing us to measure regional changes in albumin extravasation. We present he results of experiments aimed at

validating the use of this technique in locating intrarenal sites of action *in vivo*. Our method was then used to characterize events following the acute infusion of exogenous BK and selective antagonists as well as endogenous kinins using two different types of ACE inhibitors. Finally, we highlight the potential value of this model in renal function studies by presenting results obtained in a classic pathophysiological model characterized by localized changes in vascular permeability; renal ischemia-reperfusion injury. The novel data obtained gives insight into the time course of events that may ultimately lead to renal failure.

METHODS

2.1 EXPERIMENTAL TECHNIQUES

2.1.1 Surgical preparation for clearance studies

Male mongrel dogs (15-30 kg), 24 hour food restricted but allowed water *ad libitum*, were anesthetized with sodium pentobarbital (BDH Chemicals, 25 mg/kg IV) and a tracheal tube was inserted to allow free breathing. The animals were surgically prepared for clearance studies by cannulating the femoral artery for plasma sampling and monitoring cardiovascular parameters (E&M line core P-1000 servo-null transducer and E&M Physiograph-six recorder). The femoral vein was cannulated to allow volume replacement by infusing a saline solution (0.9% NaCl, 5cc/min: Buchler Instruments peristaltic pump) containing markers for evaluating glomerular filtration rate and renal plasma flow (inulin and para-aminohippurate; 2.0 and 0.4 mg/kg bolus, 0.03 and 0.006 mg/kg/min sustained infusion, respectively). Ureters were cannulated with polyethylene tubing (Intramedic, PE-160) placed near the renal pedicle to allow easy urine flow and complete collection of samples.

2.1.2 Additional preparation for intrarenal hormone infusion

In certain experiments, in addition to preparation for clearance studies the left renal artery was cannulated using a modified 21G needle and polyethylene tubing (Intramedic PE-50). This allowed us to

position a perforated section of tubing in the renal artery. One end of the tubing was occluded and the other was connected to a three way coupling. The tubing was kept patent by constantly infusing vehicle solution (Buchler constant infusion pump; 0.3 ml/min). Renal artery pressure was monitored (E&M line core P-1000 servo-null transducer and E&M Physiograph-six recorder) via the three way coupling on the infusion line.

2.1.3 Additional preparation for renal ischemia and reperfusion

In certain experiments, animals were prepared for renal ischemia and reperfusion of the left kidney by placing a silk band and rigid plastic tube to form a noose around the renal artery. This was installed loosely during surgery and allowed us to cut off blood flow to the kidney when desired without reopening the abdomen. At the end of the ischemia period the tube was removed, leaving behind the silk band, thereby relieving any obstruction to blood flow.

2.1.4 Clearance studies

Surgical preparations took about 20-30 minutes and was followed by a 60-minute equilibration period. Experiments commenced once left and right urine volumes appeared comparable and stable over three consecutive 10 minute control periods (<10% difference between collection periods). Also, stable systemic and renal average blood pressure during this time had to fall within 130±10 and 90±10 mm Hg respectively during this time. Urine from left and right kidneys

was always collected in consecutive 10 minute periods, blood samples were taken at the midpoint of each urine collection periods. Urine volume, blood pressure and heart rate were recorded.

Blood samples were immediately centrifuged, the plasma decanted and frozen together with urine samples (-20°C) until analysis. Inulin, para-aminohippurate concentrations were determined using colorimetric methods and sodium levels were assayed by flame photometry. Urine flow (UV) is expressed in ml/min and sodium excretion (U_{Na}+V) in μEq/min. Renal hemodynamic parameters are assessed from the clearance values of inulin (C_{inulin}) for GFR and PAH (C_{PAH}) for RPF, both derived from the ratio of their urine excretion rates and plasma concentration.

2.1.5 Albumin-EB binding under different pH or osmolarity

To evaluate potential changes in EB and albumin binding, the absorbance spectrum of solutions containing 0.002% EB either alone or with bovine serum albumin (in a molar ratio of 1: 4.8) at varying pH or osmolarity were measured. Solution pH was adjusted using 1-4 N HCl and a glass electrode pH meter (Corning pH meter 125). Osmolarity was adjusted using a mixture of NaCl and urea in a 1:1 osmotic ratio (i.e. 1 : 1.86 molar ratio, respectively) and an osmometer (Advanced Instruments micro-osmometer 3MO). The absorbance of different solutions was recorded by spectrophotometer with tracing and integrating capability (Perkin-Elmer Spectrophotometer Lambda

5) providing hard copy printouts of the absorbance spectrum between 565 and 645 nm and a value for the maximum absorbance frequency.

2.1.6 Measurement of vascular volumes (^{51}Cr marked red blood cell distribution volume).

Anesthetized male mongrel dogs were prepared as if for clearance studies (see section 2.1.1). The left renal artery and vein were exposed carefully, to minimize damage to surrounding tissue, and a noose fashioned from a 1 cm wide silk band and rigid polyethylene tubing was loosely fitted around them. The tubing and silk band were long enough to allow exteriorization through the abdominal incision, thereby enabling vessel occlusion without further intrusion. After a 60 minute recovery, when a stable urine flow from both kidneys was observed over three consecutive 10 minute periods, a 20 ml sample of whole blood was taken and centrifuged (5000 RPM for 10 minutes) to allow isolation of red blood cells (RBC). These were then incubated for 60 minutes at 27^{0}C in the presence of 40 µCi ^{51}Cr. RBC were then washed and centrifuged twice with isotonic saline to remove unbound ^{51}Cr. Marked RBC (4-5 ml) were prepared for injection by suspending them in enough isotonic saline to achieve systemic hematocrit values. One ml of RBC was saved to determine its radioactivity.

Marked RBC were injected via the femoral vein and allowed to circulate for 30 minutes before sacrifice. Just before sacrifice, blood hematocrit values were measured and a 10 ml sample of whole blood was retrieved from both the femoral artery and vein. The renal artery

and vein of the left kidney were then completely occluded and the kidneys were rapidly excised. The left kidney was immediately frozen in liquid nitrogen. The right kidney was decapsulated and the medullary crest exposed thereby allowing the kidney to drain freely for 1-2 minutes before freezing.

Radioactivity of marked RBC, venous and arterial whole blood as well as both kidneys was measured using a gamma counter (Canberra, series 20) with a window between 256 and 367 with a peak at 320. Radioactivity is measured in counts per minute (CPM) and corrected for background noise. Kidneys were kept frozen ($^{-}$ 70^0 C) so that vascular fluid remained in place. The kidneys were then dissected, using a band saw in a cold room (4^0 C), to isolate tissue from the cortex (CTX), outer medulla (OM), inner medulla (IM) and papilla (PAP) while ensuring that samples remained frozen. Tissue from the same zones, recovered from all five dogs, was pooled for both kidneys then weighed and the radioactivity measured.

Total vascular volume was calculated as the distribution volume of ^{51}Cr RBC by dividing the known quantity of ^{51}Cr injected by its plasma concentration at the time of sacrifice. Preliminary tests showed no free ^{51}Cr in the plasma fraction of whole blood. Whole kidney vascular volume was determined by dividing the measured CPM of the organ by that of a measured quantity of whole blood (CPM/ml). A calculation of the percent remnant vascular volume in the drained kidney is derived from the difference in radioactivity between left and right kidney. Absolute vascular volumes cannot be determined for different renal zones since axial concentration of blood is known to occur and precise regional hematocrit values are as of yet unknown.

However, the percentage of remnant vascular volume can still be estimated using the differences in regional radioactivity as for whole kidneys since hematocrit values for each zone should be the same for either kidney (this will be discussed in greater detail later).

2.1.7 Albumin extravasation in renal tissue using EB

To evaluate the extravasation of albumin in renal tissue a solution of EB (0.225 g/kg, in 50cc 0.9% saline solution) was injected via the femoral vein and allowed to circulate for one minute. The animals were then sacrificed by severing the aorta and vena cava above the renal arteries so as to reduce blood pressure evenly in both kidneys. Kidneys were rapidly excised and dissected to allow maximal vascular drainage of tissue. In all cases two tissue samples (0.1 to 0.3 g, wet weight) of CTX, OM, IM and PAP were then immediately dissected from each kidney (Figure 4). EB dye was extracted from one set of tissue samples with formamide (FISHER: 4 ml/g wet tissue, for 24 hours) and its concentration evaluated by spectrophotometry (620 nm, Titertec Multiscan M.C., type 320) using known absorbance values for a range of EB concentrations. The ratio of wet/dry tissue weight was determined, as a measure of tissue water content, from the other set of samples and is reported. These ratios are also used so that results obtained for tissue EB content can be expressed in µg EB/g dry tissue. As a final technical note, in validation studies (data not shown) we have demonstrated that urinary EB content is virtually undetectable (less than 0.1% of that in the plasma) and that no

absorbance values were observed for formamide extract of renal tissue without EB.

2.2 PROTOCOLS

2.2.1 Functional effects of bradykinin during water diuresis and urea load

In these experiments animals were prepared for clearance studies and for intrarenal hormone infusion. In water diuresis animals (N=5) the 0.9% NaCl, inulin and PAH infusion solution was replaced with one containing 0.45% NaCl and the markers 30 minutes after surgery. In these animals a variable amount of time (1-3 hours) elapsed before urinary specific gravity was reduced to levels below 1.005. Urea loaded dogs (N=7) received a bolus injection of urea (0.18 g/kg) in 50 cc of water made isotonic with NaCl. Furthermore, the standard 0.9% NaCl and marker solution was replaced with one containing 0.12 g/kg/l of urea also made isotonic with NaCl. These doses aimed to achieve and maintain plasma urea levels between 50-60 mg%. Intrarenal bradykinin infusion (0.05 µg/kg/min) was initiated immediately after three consecutive control periods and was maintained for 30 minutes. The dose of BK we use is the maximum possible which does not alter systemic cardiovascular parameters (heart rate and blood pressure) or contralateral renal function. In fact, this dose is comparable to that used by others (THOMAS *et al.*, 1982). Three more sampling periods followed after stopping the hormone infusion.

We also measured the urea content of plasma and urine as well as urine osmolarity (by colorimetric and freezing point depression methods respectively). The relative osmolar contribution of urea and NaCl were calculated using their respective urinary concentrations (for

NaCl we used Na+ excretion values and corrected using the dissociation constant for NaCl)

2.2.2 Albumin-EB binding under different pH or osmolarity

Triplicate measurements were taken of EB solutions either with or without albumin with a pH value of 7.4 and at 286 mOsm. Other solutions were measured containing EB and albumin with pH values between 4.0 and 7.4 and osmolarities from 286 to 1200 mOsm to mimick conditions that can be encountered in the renal medulla.

2.2.3 Systemic and renal vascular volumes (efficiency of renal vascular draining)

The experimental procedures described in section 2.1.7 were carried out on five male mongrel dogs weighing between 20 and 22.5 kg over a period of four consecutive days. Whole kidneys, marked RBC and whole blood samples were stored together at -70^{0}C until analysis. The radioactivity of these samples was measured on day 7 while ensuring that they remain frozen. Whole kidneys were then dissected, while frozen, over the course of the next two days. The radioactivity of pooled tissue samples was determined on day 10 and that of RBC and whole blood measured again at this time to allow for ^{51}Cr decay.

2.2.4 Renal function and albumin extravasation studies in control, water diuresis and hypertonic saline loading dogs

In these studies, both renal function and albumin extravasation was measured in the same animals. Functional parameters were measured to evaluate the state of the animal at the time when albumin extravasation measurements are taken. To establish normal (control) albumin extravasation values, untreated animals (N=7) were prepared for and underwent renal clearance studies, the procedures for albumin extravasation measurement were initiated immediately after the third urine sampling period.

Water diuretic animals (N=5) were prepared as described in section 2.2.1, Once urine specific gravity less than 1.005 was attained in both kidneys urine sampling and albumin extravasation measurement was initiated as with untreated animals.

To study the effects of hypertonic saline infusion dogs (N=5), procedures were exactly as for water diuresis but instead a 2.0% NaCl marker solution was substituted for the isotonic one 30 minutes after surgery. Urine collection and albumin extravasation measurement were performed as for the untreated and water diuresis animals but was initiated after two hours of infusion.

2.2.5 Albumin extravasation studies following the intrarenal infusion of bradykinin alone and concurrently with selective receptor antagonists

In these experiments assessment of functional responses had been previously established (and are described in the introduction) so measurement of albumin extravasation in response to peptide hormone infusion was performed on three separate series of animals. Animals were prepared for clearance studies and for intrarenal hormone infusion as described above. Hormone infusion and albumin extravasation measurement was initiated after three consecutive 10 minute urine samples had been collected to establish baseline renal functional parameters before hormone infusion. In the first series (Group 1, N=7) animals received BK alone (0.05 μg/kg/min delivered at 0.3 ml/min) for 5 minutes. In the second and third series of experiments (Groups 2 and 3, N=5 & 6, respectively) the three control periods are followed by a 10 minute infusion of either a B_1 or a B_2 kinin receptor antagonist (2.0 μg/kg/min anti B_1 or anti B_2, at 0.3 ml/min) in the left renal artery followed by the infusion of a solution of BK and antagonist (0.05 μg/kg/min BK with 2.0 μg/kg/min anti B_1 or anti B_2, at 0.3 ml/min) over 5 minutes.

Bradykinin, B_1 receptor antagonist ([Leu^8]-des-Arg^9-BK) and B_2 receptor antagonist (D-Arg^0, [Hyp^3, D-Phe^7]-BK) were synthesized and provided graciously by D. Regoli (Université de Sherbrooke, Dépt. Pharmacologie). The selectivity and affinity of these antagonists have been systematically assayed and reported previously (REGOLI *et al.*,

1990). The doses of anti-B_1 and anti-B_2 used in these experiments were determined following tests in four dogs in which we infused different doses of the substances in the left kidney (0.01, 0.05, 0.1, 1.0, 2.0, 10.0 µg/kg/min; for 10 min). The infusion of 10.0 µg/kg/min of either antagonist caused erratic responses in both systemic cardiovascular parameters and left renal hemodynamics, while 2.0 µg/kg/min did not. No other intrinsic effect was observed with the latter dose. Others have also shown that high doses of receptor antagonist can effectively block exogenous BK without initiating any physiological response of their own (MULINARI *et al.*, 1989, REGOLI and BARABÉ, 1980).

2.2.6 Renal function and albumin extravasation profiles during equipotent doses of Captopril and Perindopril

The effects of Captopril or Perindopril were determined in different groups of 5 untreated dogs prepared for clearance studies and intrarenal hormone infusion. Doses used were 0.8 and 0.08 µg/kg/min, respectively, infused into the left renal artery for thirty minutes. Intrarenal infusion of Captopril at this dose, in normal dogs, has been reported to have little or no direct effect on renal function (WILCOX, 1988). The dose of Perindopril reflects it's ten fold difference in potency with regards lowering blood pressure (I.R.I.S, 1983). Measurement of albumin extravasation was initiated immediately after the third urine sampling period.

2.2.8 Changes in renal function and albumin extravasation after 30 or 60 minutes of post-ischemia reperfusion

Thirteen dogs were prepared for clearance studies and renal artery occlusion. After three consecutive control collection periods, blood flow in the left renal artery was interrupted by drawing firmly on the silk band of the noose. Urine collection continued for the right kidney during the thirty minute occlusion period. Reperfusion of the left kidney ensued for either 30 (N=6) or 60 minutes (N=7) at which point albumin extravasation was measured.

2.2.9 Data analysis

Data analysis of renal function data considers intra- and intergroup changes in both kidneys before and during treatment. Variance analysis and Duncan grouping, where applicable, were used in the following comparisons: **intragroup** 1) all control values, 2) left kidney control values, 3) right kidney control values, 4) left kidney values for each experimental period versus the mean control value, 5) right kidney values for each experimental period versus the mean control value, 6) left versus right kidney values for each experimental period; **intergroup** 7) all control values of the groups, 8) left kidney control values of the groups, 9) right kidney control values of the groups, 10) left kidney values of the groups for each experimental period and their mean control values, 11) right kidney values of the groups for each experimental period and their mean control values. Left and right kidneys are considered paired and independent.

Significance is denoted for p values of <0.05 or <0.01 as specified in each diagram and table used in the text.

Significance of the differences in maximal absorbance frequency of EB solutions of varying composition was determined by Duncan grouping (comparing values in similar groups to control values), mean values of triplicate readings were used.

Variance analysis was used to detect differences between whole blood radioactivity, hematocrit as well the systemic and renal vascular volumes (expressed as net value or per unit weight). Since there was insignificant variation between dogs in these parameters we felt justified in pooling similar tissue for left and right kidneys from all five dogs to minimize errors due to nonspecific background noise when recording radioactivity from these small samples. Therefore only one net value is reported for the percent vascular drainage of different renal zones.

Intra- and intergroup data analysis of tissue EB content (i.e. albumin extravasation) and wet/dry tissue ratios considers the mean values ±1 standard error for each zone of both left and right kidneys in each group. Variance analysis and Duncan grouping was used in intragroup comparison between the four zones of each kidney as well as between similar zones of left and right kidneys. Values for homologous zones of the left or right kidneys in the various groups were also compared. Left and right kidneys were considered paired and independent. Significance is denoted for *p* values of <0.05 or <0.01.

RESULTS

3.1 Functional effects of bradykinin during water diuresis and urea load

Systemic and renal blood pressure were within the limits of normalcy during the control periods of all these experiments. As seen during pilot studies the dose of bradykinin used did not significantly alter systemic or renal blood pressure. The results obtained from these experiments are presented in Figures 1 & 2 as well as in Table I. For comparison, results of previously reported experiments on the effects of bradykinin alone in normal animals are included in Table I.

Water diuresis induced by systemic infusion of 0.45% saline solution caused bilateral sustained increase in UV and $U_{Na}+V$ without altering renal hemodynamic parameters. Values obtained during the three control periods are not different from those reported for normal animals in section 3.4. The infusion of bradykinin into the left renal artery caused significant and sustained unilateral increases in both UV and $U_{Na}+V$. Urine flow (ml/min) in the left kidney increased from an average value of 5.27±0.20 to a maximum of 6.5±0.79 ($p<0.01$) in the first 10 minutes and remained significantly elevated (6.1±0.7, $p<0.05$) over the remaining 20 minutes of infusion. In the same fashion, $U_{Na}+V$ (μEq/min) increased from 254±20 to 342±40 ($p<0.01$) and remained significantly elevated (325±42,$p<0.05$) for the duration of the infusion. These effects were immediately reversed upon terminating bradykinin infusion.

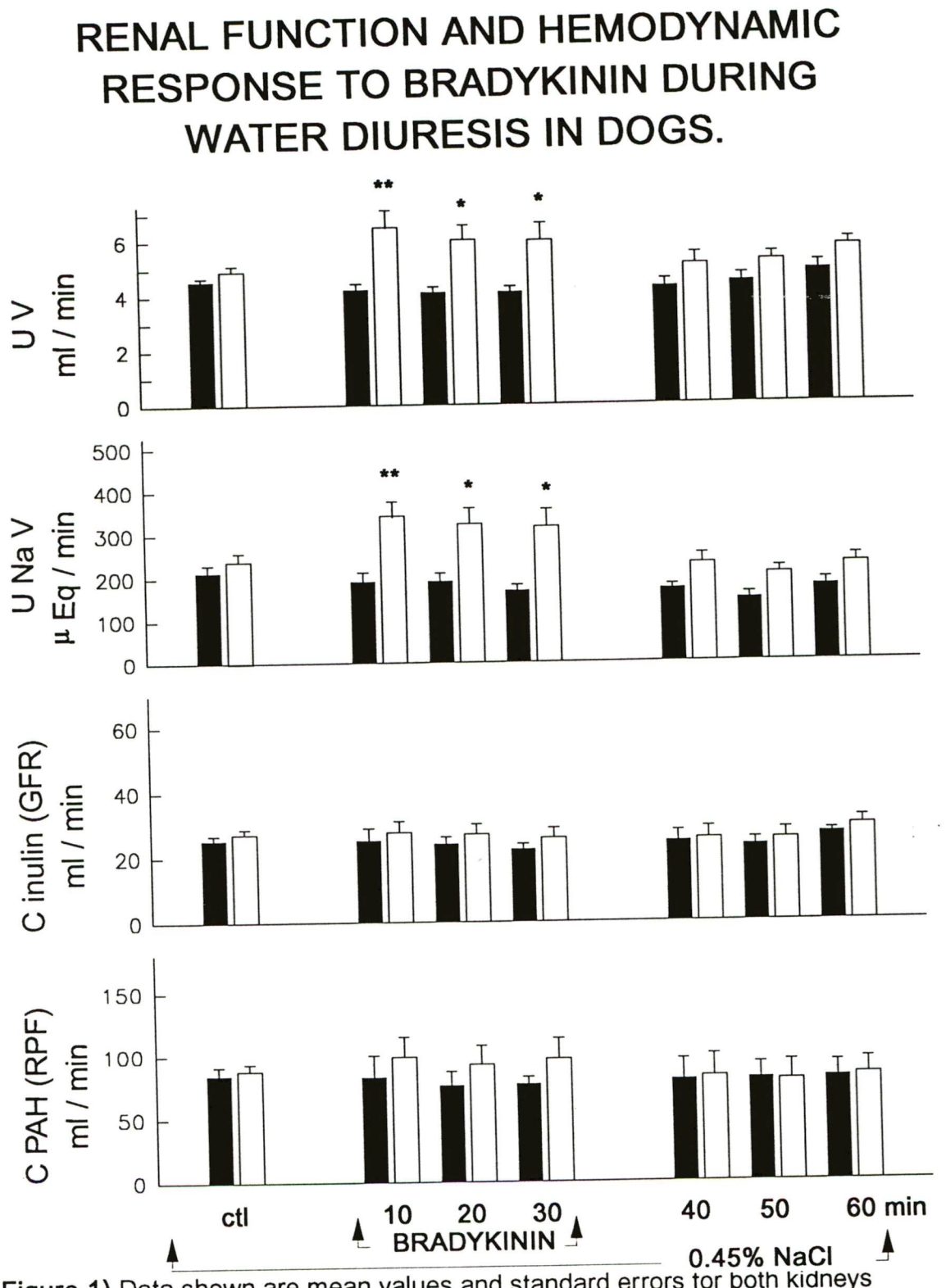

Figure 1) Data shown are mean values and standard errors for both kidneys before, during and after bradykinin infusion in the left kidney of 5 dogs during water diuresis. Solid bars indicate right kidney and open bars the left The symbols * & ** indicate $p < 0.05$ & 0.01, respectively, versus control values (see text for details).

RENAL FUNCTION AND HEMODYNAMIC RESPONSE TO BRADYKININ IN UREA LOAD DOGS.

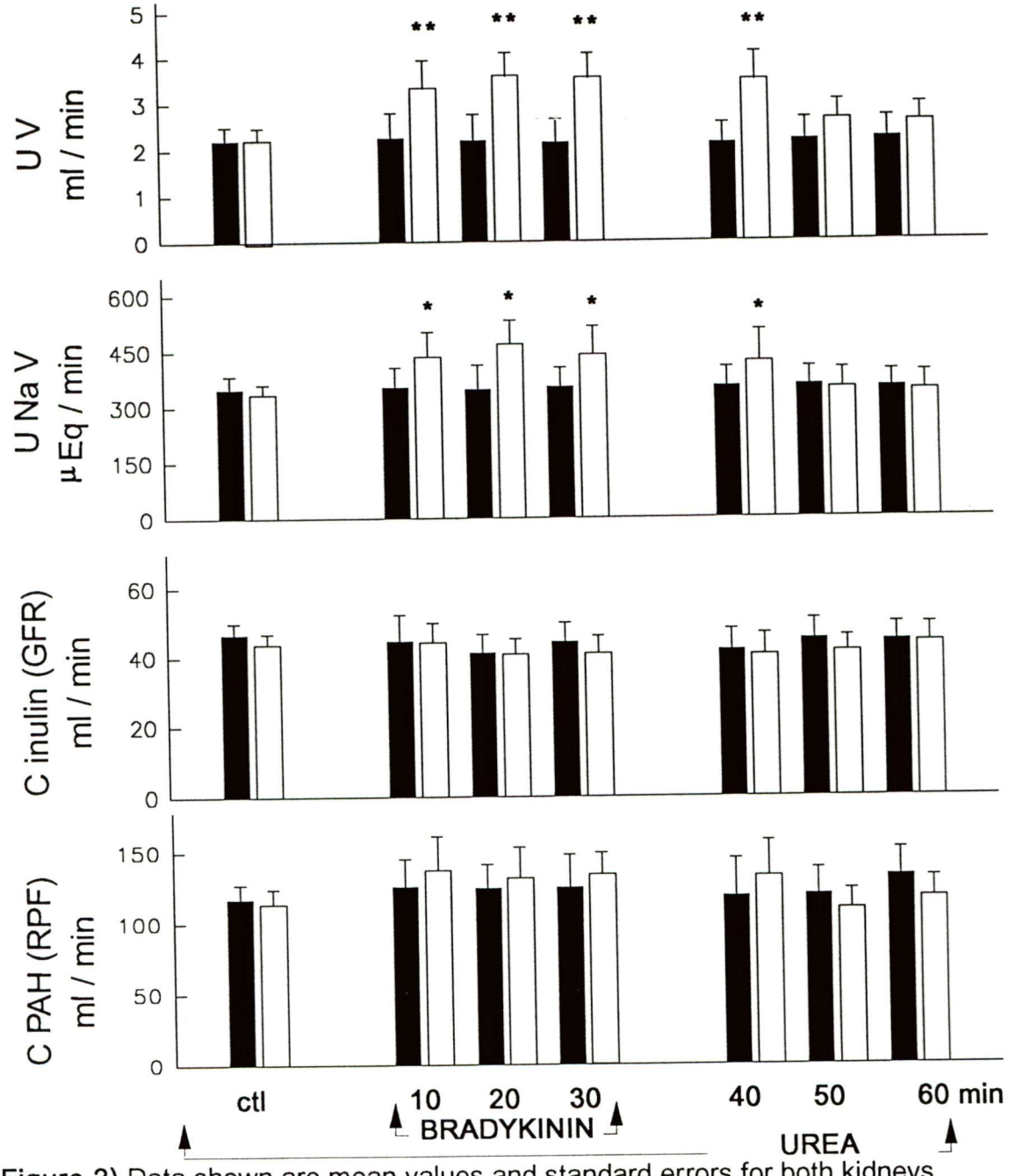

Figure 2) Data shown are mean values and standard errors for both kidneys before during and after bradykinin infusion in the left kidney of 7 urea loaded dogs. Bars and symbols as in Figure 1 (see text for details).

Renal hemodynamic parameters were unaltered by bradykinin under these conditions. The infusion of bradykinin did not significantly alter baseline urine osmolarity in the left kidney (115±7 mOsm/l) or the excretion of urea (6.5±0.5 mg/min). The contribution to urine osmolarity of Na+ and urea during the control periods were calculated to be 98±7 and 22±2 mOsm/l, respectively, and was not significantly changed by the infusion of bradykinin.

Urea infusion increased and maintained plasma urea levels to an average of 68±8 mg% over the course of the experiment. This caused a significant and sustained bilateral increase in both UV and $U_{Na}+V$ ($p<0.01$) when compared to values in normal animals (see Table I.). Bradykinin in the left renal artery caused an immediate and sustained significant increase in UV that persisted for the duration of the infusion period and for the first 10 minutes following the end of the infusion ($p<0.01$, for all four periods). Flow rates increased from baseline values of 2.14±0.26 to a maximum of 3.59±0.61 ml/min in the second period of infusion. Similarly, $U_{Na}+V$ for all three infusion periods and the first recovery period was significantly increased by bradykinin ($p<0.05$). Left kidney $U_{Na}+V$ increased from 333±27 to a maximum of 468±44 in the second period of infusion.

Renal hemodynamic parameters, C_{inulin} and C_{PAH}, were unaltered by bradykinin under these conditions. However, the infusion of bradykinin did significantly reduce urine osmolarity in the left kidney from a baseline value of 550±60 to a minimum of 330±44 mOsm/l ($p<0.01$) in the last period of infusion. Urine osmolarity was not different from baseline values within 20 minutes of stopping hormone infusion. The excretion of urea under these conditions was unchanged

from baseline values (16.0±1.8 mg/min) by bradykinin. The contribution to urine osmolarity of Na+ and urea during the control periods were calculated to be 310±37 and 125±23 mOsm/l, respectively. During the infusion of bradykinin, a significant decrease in these parameters to an average of 260±20 ($p<0.01$) and 81±17 ($p<0.05$) mOsm/l was observed. Interestingly, under urea load, an osmotic gap is observed in control conditions such that the combined osmolar contribution calculated for urea and sodium is far less than measured urine osmolarity. This difference of 115 mOsm/l is corrected by the infusion of BK.

THE EFFECT OF BRADYKININ ON THE OSMOLAR COMPOSITION OF URINE IN NORMAL, WATER DIURETIC AND UREA LOADED DOGS:

Clearance and calculated values for sodium and urea.

	NORMAL N = 6		W. DIURESIS N = 5		UREA LOAD N = 7	
	CTL	BK	CTL	BK	CTL	BK
U V; ml / min	**0.4**±0.2	**1.7**±0.5**	**5.0**±0.2	**6.1**±0.7*	**2.1**±0.3	**3.4**±0.7 **
U Na^+V; µEq / min	**55**±13	**135**±25**	**254**±20	**325**±42 *	**333**±27	**450**±40 *
U urea V; mg / min	**5.0**±0.4	**6.0**±0.4	**6.5**±0.5	**6.5**±0.4	**16.0**±1.8	**7.0**±1.4
Osm. total; mOsm	ND		**115**±7	**120**±10	**550**±60	**360**±60 **
Osm. Na^+; mOsm	**217**±8	**156** ±10**	**98**±7	**106**±11	**310**±37	**260**±20 **
Osm. urea;mOsm	**166**±14	**55**±8**	**22**±2	**18**±6	**125**±23	**81**±17 *

Table I) Mean values of three consecutive 10 minute sampling periods from the left kidney are presented. Values represent conditions before (CTL) and during bradykinin (BK) infusion in the left kidney. Significant difference from control is indicated by * & ** for $p <$ 0.05 & 0.01 respectively (see text for details).

3.2 Albumin-EB binding under different pH or osmolarity

The results of experiments aimed at determining possible changes in binding of EB with albumin under varying pH and osmolarity are summarized in Tables II-a & II-b. In the absence of albumin, the maximal absorbance frequency of an EB solution, at pH 7.4 and an osmolarity of 286 mOsm, was 603.6 nm. When albumin was present in an identical solution the maximal absorbance frequency was significantly different ($p<0.01$) at 614.8 nm. As seen in the table neither acidification or increased osmolarity of the solution caused the maximal absorbance frequency to revert to that observed for EB in the unbound state.

3.3 Systemic and renal vascular volumes (efficiency of renal vascular draining)

Animals in these experiments had a mean blood pressure values within the 130±10 mm Hg limit during the three collection periods. Clearance studies during the 30 minutes before sacrifice revealed no difference between left and right kidneys and a mean urine flow of 0.8±0.2 ml/min per kidney was observed. Similarly, sodium excretion was 165±30 μEq/min, C_{inulin} was 33±5 ml/min and C_{PAH} was 109±14 ml/min for each kidney. This was not different from control values established by us and reported in Section 3.1.

Radioactivity measurements of marked RBC reveal that an average of 3.79±0.12 $x10^5$ CPM was injected into each animal. Blood

THE EFFECT OF VARYING SOLUTION pH AND OSMOLARITY ON EVANS BLUE DYE BINDING TO ALBUMIN

a) Varying pH

Albumin	pH	mOsm	Max. abs. frequency (nm)
-	7.4	286	603.6 **
+	7.4	286	614.8
+	6.0	286	614.8
+	5.1	286	616.2
+	4.1	286	618.8

b) Varying osmolarity

Albumin	pH	mOsm	Max. abs. frequency (nm)
-	7.4	286	603.6 **
+	7.4	286	614.8
+	7.4	518	614.8
+	7.4	763	614.8
+	7.4	1154	614.8

Table II-a, b) Mean values of triplicate absorbance readings for solutions of varying pH and osmolarity. In this case, the symbol ** shows a value significantly ($p<0.01$) different from that of the others (see text for details).

SYSTEMIC AND RENAL VASCULAR VOLUME MEASUREMENTS USING ^{51}Cr RBC:

Remnant vascular volume in the drained canine kidney.

a) MEASURED VALUES:

Total body weight	18.4 ± 0.9 kg
Hematocrit	38.7 ± 1.1 %
Whole blood radioactivity	190.9 ± 16.0 CPM/ml

b) CALCULATED VASCULAR VOLUMES:

Total vascular volume	1966.0 ± 132.2 ml
Total vascular volume/ kg	107.8 ± 5.7 ml/kg
Blood EB concentration	2.2 mg/kg
LK vascular volume	1.82 ± 0.28 ml
RK vascular volume	0.16 ± 0.04 ml

c) REMNANT RENAL VASCULAR VOLUME IN DRAINED (RIGHT) KIDNEY:

Whole kidney	8.7%
Papilla + inner medulla	20.4%
Outer medulla	19.0%
Cortex	6.5%

Table III-a, b, c). Summary of results from experiments (N = 5) aimed at determining the extent of renal vascular drainage when sampling renal tissue as described in the text. Left kidneys (LK) were clamped and frozen to maintain blood volume and distribution, right kidneys (RK) were drained. All but the three values reported are the mean ±1 standard error, the last three values in table I-c result from pooling tissue samples (see text for details).

hematocrit values from the femoral artery and vein never differed by more than one unit (%) just before sacrifice, the mean for the group was 38.7±1.1 % (see Tables III-a,-b, & -c). The average between arterial and venous whole blood radioactivity was used to calculate total blood volume in each dog and averaged 190.9±16 CPM/ml. Total blood volume was calculated for each dog, the mean was 1966.0±132.2 ml per dog or 107.8±5.7 ml/kg.

Calculation of the renal vascular volume in the clamped (left) kidney shows that they contained 1.82±0.28 ml of whole blood. Values for the drained (right) kidneys reveals near complete drainage of the renal vasculature with only 0.16±0.04 ml remaining. This remnant vascular volume amounts to 8.7±2% of the total renal vascular volume. The percentage of remnant vascular volume for each zone was calculated from the difference in CPM of pooled tissue samples. These were 20.4, 19.0 and 6.5% for the inner medulla (with papilla), outer medulla and cortex respectively. It should be recalled that, by mass, the largest fraction of renal tissue is made up of the cortex while that of the papilla is minimal.

Using the values calculated here and knowing the quantity of EB injected into animals for albumin extravasation studies, we can estimate the total vascular contribution to tissue EB measurements. Approximately 4000 μg EB are contained in the renal blood vessels of the whole kidney when full and < 340 μg EB when drained.

3.4 Renal function and albumin extravasation studies in control, water diuresis and hypertonic saline loading in dogs

Average systemic blood pressure over the three collection periods were within the limits of normalcy. The results of renal function studies taken over three consecutive 10 minute clearance periods show some significantly different profiles (Figure 3). With regards to UV rates, a mean average of 0.58±0.09, 1.90±0.17 and 3.24±0.41 ml/min was recorded for normal, salt loaded and water diuretic dogs respectively. Values for both salt loaded and water diuretic dogs are significantly greater ($p<0.01$) than those of untreated animals (considered here as control values). Sodium excretion values were 117±11, 349±34 and 198±11 for untreated, salt loaded and water diuresis dogs respectively. Values from both salt loaded and water diuresis animals showed a significant difference from control ($p<0.01$). Values for renal hemodynamics, C_{inulin} and C_{PAH} (ml/min), was 29.1±1.3 and 88.5±8.0 for untreated, 29.8±1.2 and 87.9±3.7 for salt loaded and finally 33.3±2.0 and 97.7±7.7 for water diuresis animals. Evidently, there was no significant differences in renal hemodynamic parameters between these groups.

When cut in a sagittal plane, it is immediately apparent that there is a heterogeneous distribution of EB dye in the kidney which results in three distinct areas of coloration (Figure 4). Note that no discriminating feature allows the differentiation between IM and PAP tissue. In these studies PAP tissue refers to the medullary crest. As expected, no difference was found between left and right kidneys in

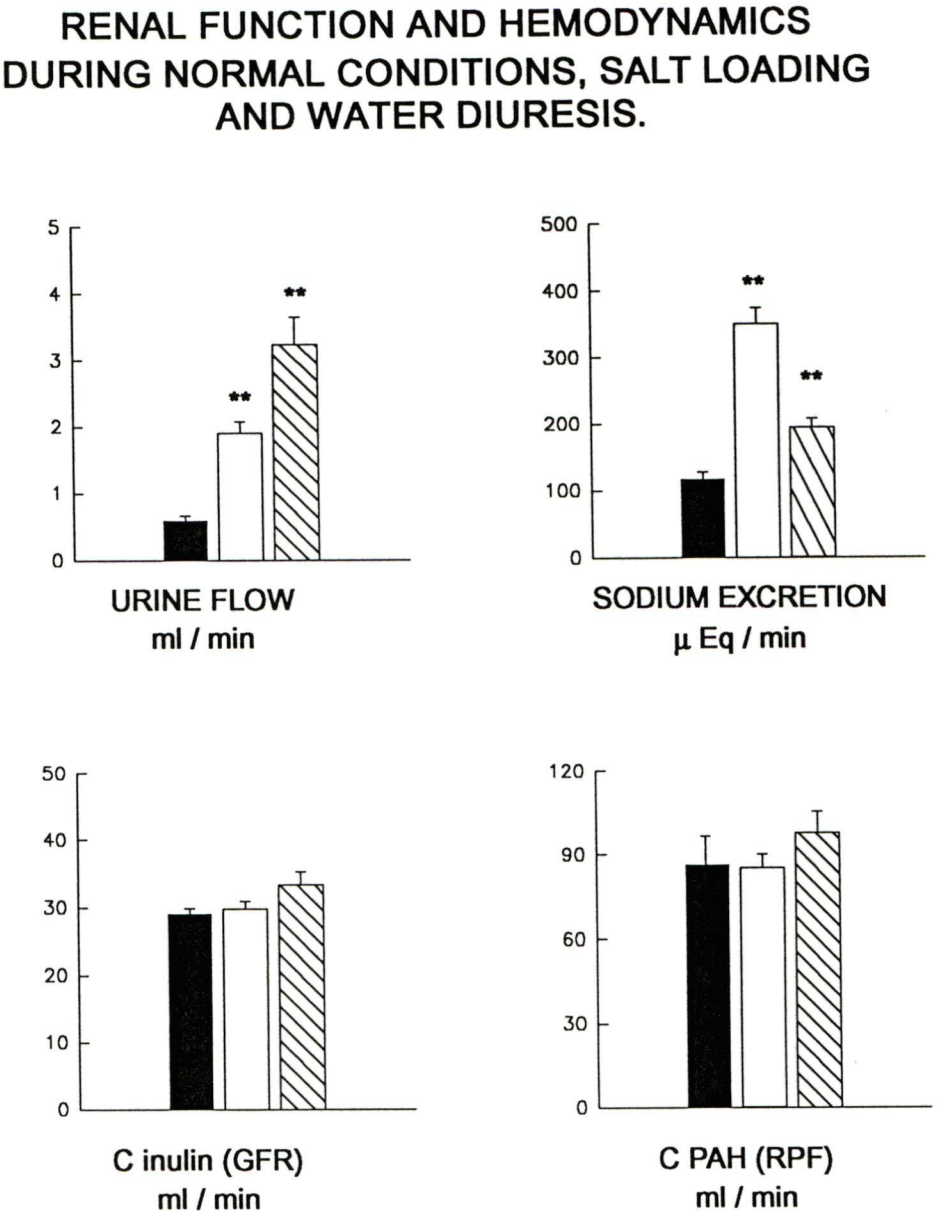

Figure 3) Data represents the mean values and standard errors for three consecutive 10 min. periods. Symbols as in Figure 1. Values for control, hypertonic saline and water diuresis are represented by solid, open and striped bars respectively (see text for details).

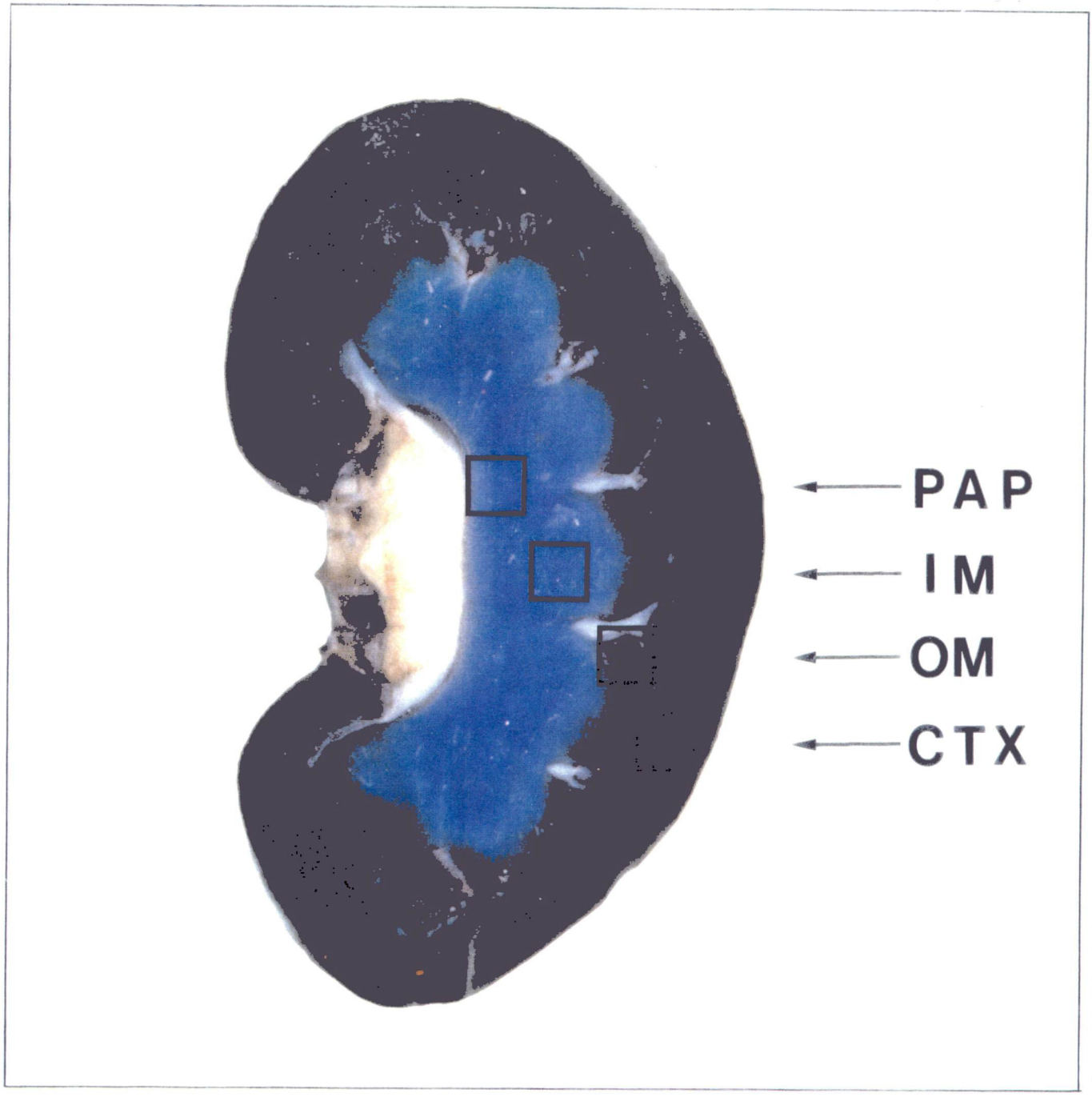

Figure 3) A sagital cut of the normal canine kidney showing the heterogeneous distribution of Evans Blue dye in the different zones.

the regional distribution of EB in these animals since both were subjected to the same conditions. Therefore in this section we report the mean combined values derived from both kidneys.

The EB content of renal tissue from normal animals was 131±8, 403±28, 750±30 and 740±47 μg EB/g dry tissue for the CTX, OM, IM and PAP respectively (Table IV). Wet/dry tissue ratios for the respective zones are 4.43±0.06, 5.14±0.13, 6.96±0.30 and 6.20±0.35 (Table V). Note that EB and tissue water content for the PAP and IM are not different from each other but that both are significantly different from those of the other two zones ($p<0.01$). This relation holds true for practically every experiment done using this technique and will not be reiterated (exceptions to the rule will be clearly indicated). The values from these experiments are considered as control values in all subsequent experiments.

Tissue EB values (μg EB/g dry tissue) were 131±10, 510±35, 959±62 and 764± 65 in salt loaded dogs and 145±10, 576±40, 936±49 and 833±39 during water diuresis dogs for the CTX, OM, IM and PAP respectively. These values are significantly higher than control in the OM ($p<0.01$) and IM ($p<0.01$) of salt loaded dogs and similarly for the OM ($p<0.01$), IM ($p<0.01$) and papilla ($p<0.05$) during water diuresis. Wet/dry tissue ratios (tissue water content) of the CTX, OM, IM and PAP were 4.37±0.07, 5.66±0.17, 7.88±0.22 and 7.33±0.27 for salt loaded dogs and 4.53±.08, 6.09±0.11, 8.95±.18 and 8.15±0.17 for those undergoing water diuresis. These are significantly greater than control values in the IM ($p<0.01$) and PAP ($p<0.05$) of salt loaded animals and in the OM ($p<0.01$), IM and PAP ($p<0.01$) during water diuresis. Furthermore, ratios obtained in all regions but the CTX

REGIONAL EXTRAVASATION OF ALBUMIN BOUND EVANS BLUE DYE IN THE CANINE KIDNEY:

Difference between control, hypertonic saline infusion (hypert. saline) and water diuresis.

MEAN OF BOTH KIDNEYS	CONTROL N= 12	HYPERT. SALINE N= 10	WATER DIURESIS N= 14
CORTEX	131±8	131±10	145±10
OUTER MEDULLA	403±28	510±35 **	576±40 **
INNER MEDULLA	750±30	959±62 **	936±49 **
PAPILLA	740±47	764±65	833±39 *

Table IV) Values indicate μg of Evans Blue dye per g of dry tissue. The symbols * & ** indicate *p* values <0.05 and 0.01, respectively, versus control values. N values indicate the number of kidneys studied (see text for details).

REGIONAL TISSUE WATER CONTENT

IN THE CANINE KIDNEY :

Difference between control, hypertonic saline infusion (hypert. saline) and water diuresis.

MEAN OF BOTH KIDNEYS	CONTROL N= 12	HYPERT. SALINE N= 10	WATER DIURESIS N= 14
CORTEX	4.43±0.06	4.37±0.07	4.53±0.08
OUTER MEDULLA	5.14±0.13	5.66±0.17 *	6.09±0.11 **
INNER MEDULLA	6.96±0.30	7.88±0.22 **	8.95±0.18 **
PAPILLA	6.20±0.35	7.33±0.27 *	8.15±0.17 **

Table V) Values indicate the ratio of wet over dry tissue weight. N values indicate the number of kidneys studied. Significance symbols as in Table IV (see text for details).

of animals undergoing water diuresis are significantly greater than those of salt loaded animal ($p<0.01$).

3.5 Albumin extravasation studies following the intrarenal infusion of bradykinin alone and concurrently with selective receptor antagonists

No significant changes in systemic or renal blood pressure is observed in these experiments. Results of these studies are compared to those from normal (control) animals presented in section 3.4 and are summarized in tables VI and VII. For clarity, values of individual kidneys from control experiments are presented. The results obtained when bradykinin alone was infused, reveal a significant unilateral increase in the EB content of the left cortex versus the right (201±7 and 145±11 μg EB/g dry tissue, respectively, $p<0.01$). This difference holds true when the left kidney cortex is compared to control values. In the other zones, no difference between left and right, or respective control values are found. Interestingly, whereas left cortex EB content in this group increased by approximately 40%, the wet/dry tissue ratio in this zone was unchanged. In fact, none of the values in this group are significantly different from their corresponding control values.

No significant difference was found when comparing the distribution of EB and water content between respective zones of the control group those of animals infused with bradykinin plus the B_1 or B_2 kinin antagonist. The left kidney CTX values for EB content in both these groups (125±6 and 138±8 μg EB/g dry tissue, respectively) are

REGIONAL EXTRAVASATION OF ALBUMIN BOUND EVANS BLUE DYE IN THE CANINE KIDNEY:

Bradykinin alone and with selective kinin receptor antagonists.

LEFT KIDNEY	CONTROL N= 6	BK N= 7	BK + ANTI-B1 N= 5	BK + ANTI-B2 N= 5
CORTEX	125±11	201±7 **	125±6	138±8
OUTER MEDULLA	398±56	477±33	414±41	404±37
INNER MEDULLA	763±51	868±85	801±80	770±68
PAPILLA	741±52	836±75	773±69	765±80

RIGHT KIDNEY	CONTROL N= 6	BK N= 7	BK + ANTI-B1 N= 5	BK + ANTI-B2 N= 5
CORTEX	136±15	145±11	119±5	149±7
OUTER MEDULLA	409±29	473±43	422±40	444±32
INNER MEDULLA	736±44	829±63	721±74	757±58
PAPILLA	739±76	746±93	802±75	796±85

Table VI) Values indicate µg of Evans Blue dye per g of dry tissue. BK indicates bradykinin, Anti-B1 and Anti-B2 are selective kinin receptor antagonists. Significance symbols as in Table IV (see text for details)

REGIONAL TISSUE WATER CONTENT

IN THE CANINE KIDNEY :

Bradykinin alone and with selective kinin receptor antagonists.

LEFT KIDNEY	CONTROL N= 6	BK N= 7	BK + ANTI-B1 N= 5	BK + ANTI-B2 N= 5
CORTEX	4.48±0.05	4.50±0.05	4.34±0.10	4.40±0.08
OUTER MEDULLA	5.10±0.19	5.28±0.19	5.54±0.12	5.41±0.09
INNER MEDULLA	7.13±0.37	6.93±0.40	7.66±0.29	7.70±0.28
PAPILLA	6.35±0.30	6.08±0.34	6.84±0.36	6.20±0.11

RIGHT KIDNEY	CONTROL N= 6	BK N= 7	BK + ANTI-B1 N= 5	BK + ANTI-B2 N= 5
CORTEX	4.37±0.10	4.42±0.16	4.48±0.05	4.40±0.07
OUTER MEDULLA	5.19±0.15	5.48±0.17	5.50±0.07	5.22±0.10
INNER MEDULLA	6.80±0.42	7.52±0.35	7.46±0.25	7.04±0.30
PAPILLA	6.05±0.52	7.21±0.48	6.72±0.47	6.70±0.52

Table VII) Values indicate ratio of wet over dry tissue weight. BK indicates bradykinin, Anti-B1 and Anti-B2 are selective kinin receptor antagonists. Significance symbols as in Table IV (see text for details).

significantly ($p<0.01$) less than observed for bradykinin alone, while water content values for this zone are equivalent. No significant difference is found between the respective values of the other zones, for either parameter. The values for right kidney cortex of dogs recieving antagonists and BK differ from each other but are not significantly different from their respective left kidney values or from right kidney values of control animals.

3.6 Renal function and albumin extravasation profiles during equipotent doses of Captopril and Perindopril

The results of these experiments are presented in Figure 5 and Tables VIII and IX. The dose of Captopril used (0.8 μg/kg/min, intrarenally) was shown by others to have no intrinsic effect of systemic blood pressure but to be effective in preventing the conversion of angiotensin I infused in the rat renal artery. In our hands, no changes in systemic and renal blood pressure or in renal function parameters were observed following Captopril infusion. However, albumin extravasation studies in these animals revealed a significant decrease ($p<0.05$) in tissue EB content of the left kidney PAP (588±66 μg EB/g dry tissue) when compared to either the contralateral kidney or to control values (786±52 and 741±52 μg EB/g dry tissue, respectively). This was not accompanied by a change in tissue water content in any of the renal zones.

Perindopril at a dose 10 times less than for Captopril, (reflecting the tenfold difference in potency for lowering blood pressure) also had no significant effect on renal function. There does appear to be a

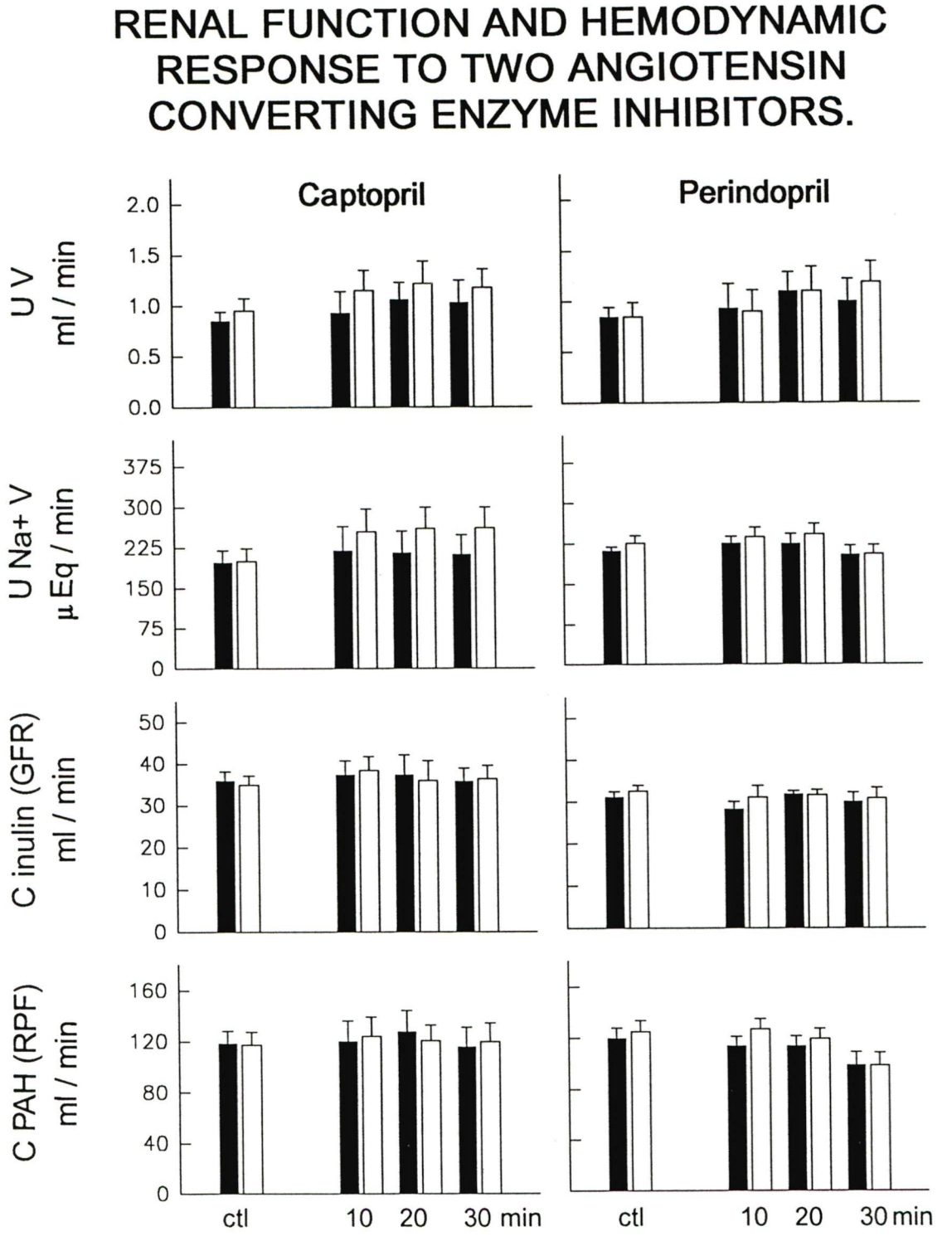

Figure 5) Data shown are mean values and standard errors for both kidneys before and during left kidney infusion of either Captopril (N=5) or Perindopril (N=5). Bars and significance symbols as in Figure 1 (see text for details).

REGIONAL EXTRAVASATION OF ALBUMIN BOUND EVANS BLUE DYE IN THE CANINE KIDNEY:

Efffect of two different angiotensin coverting enzyme inhibitors.

LEFT KIDNEY	CONTROL N= 6	CAPTO. N= 5	PERINDO. N= 5
CORTEX	125±11	113±13	140±14
OUTER MEDULLA	398±56	434±54	562±46 **
INNER MEDULLA	763±51	850±70	877±57 *
PAPILLA	741±52	588±66 *	700±38

RIGHT KIDNEY	CONTROL N= 6	CAPTO. N= 5	PERINDO. N= 5
CORTEX	136±15	115±7	142±6
OUTER MEDULLA	409±29	406±36	572±24 **
INNER MEDULLA	736±44	769±82	858±45 *
PAPILLA	739±76	786±52	696±56

Table VIII) Values indicate µg of Evans Blue dye per g of dry tissue. Capto. and Perindo. indicate left kidney infusion of Captopril and Perindopril. Significance symbols as in Table IV (see text for details).

REGIONAL TISSUE WATER CONTENT IN THE CANINE KIDNEY :

Efffect of two different angiotensin coverting enzyme inhibitors.

LEFT KIDNEY	CONTROL N= 6	CAPTO. N= 5	PERINDO. N= 5
CORTEX	4.48±0.05	4.50±0.05	4.38±0.09
OUTER MEDULLA	5.10±0.19	5.28±0.19	** 5.80±0.22
INNER MEDULLA	7.13±0.37	6.93±0.40	7.72±0.48
PAPILLA	6.35±0.30	6.08±0.34	6.94±0.37

RIGHT KIDNEY	CONTROL N= 6	CAPTO. N= 5	PERINDO. N= 5
CORTEX	4.37±0.10	4.42±0.16	4.34±0.02
OUTER MEDULLA	5.19±0.15	5.20±0.19	** 5.94±0.28
INNER MEDULLA	6.80±0.42	7.55±0.33	7.62±0.51
PAPILLA	6.05±0.52	6.18±0.30	6.52±0.27

Table IX Values indicate ratio of wet over dry tissue weight. Capto. and Perindo. indicate left kidney infusion of Captopril and Perindopril. Significance symbols as in Table IV (see text for details).

tendency towards a bilateral decrease in renal blood flow (C_{PAH}) in the final collection period but this did not reach statistical significance. Blood pressure in these animals also tended to decrease, from 131±7 to 125±9 mm Hg, but not significantly. Surprisingly, left kidney infusion of Perindopril had an effect on albumin extravasation and wet/dry tissue ratios in both kidneys. Similar significance in the increases in tissue EB content was recorded in the OM ($p<0.01$) and the IM ($p<0.05$) of both kidneys. Tissue water content was increased significantly ($p<0.01$) in the OM of both kidneys, values for the IM tended to be higher than control values but did not achieve statistical significance.

3.7 Changes in renal function and albumin extravasation after 30 or 60 minutes of post-ischemia reperfusion.

Systemic blood pressure and heart rate were not significantly altered at any point during these procedures. Renal blood pressure was reduced to zero in the left kidney during the ischemia period and returned to baseline values within the first 30 minutes of reperfusion. During this time mean renal blood pressure was higher than control values 115±15 versus 87±9 mm Hg ($p<0.05$) but was seen to oscillate variably from one animal to another.

As shown in Figure 6, no significant differences between left and right kidney values were observed during the control periods (mean of three consecutive periods). Left kidney function was obviously halted during the 30 minute renal artery occlusion and no significant changes in right kidney function were observed during this time. Renal

parameters up to and including the first 30 minutes of reperfusion are those of all 13 animals, the results for 40-60 minutes are those of the 7 dogs that underwent 60 minutes of reperfusion.

Left kidney urine flow was significantly less than control values during the initial 10 minutes of reperfusion: 0.41±0.14 compared to 0.80±0.08 ml/min ($p<0.01$). Subsequently, UV in the left kidney was fully restored. $U_{Na}{}^{+}V$ in the left kidney was significantly reduced in the first 20 minutes of reperfusion, from 161±18 to 60±21 μEq/min in the first 10 minutes ($p<0.01$), but was not different from baseline or contralateral values in subsequent periods. As with UV, the trend towards an increase in $U_{Na}{}^{+}V$ did not attain statistical significance in the right kidney.

In the left kidney, a 60 minute reperfusion period saw a partial return towards control values of C_{PAH} and C_{inulin}. In the first 10 minutes of left kidney reperfusion, C_{PAH} was reduced to 25% of control, decreasing from 90.1±7.8 to 22.6±6.8 ml/min. C_{PAH} remained significantly lowered ($p<0.01$), in spite of a gradual increase to approximately 63% of control values after 60 minutes of reperfusion. The changes in C_{inulin} paralleled those for C_{PAH}. Thus, during the initial reperfusion period, C_{inulin} in the left kidney was reduced by 75%, from 34.2±2.1 to 9.0±2.9 ml/min, and gradually increased to 54% of control after 60 min of reperfusion, thereby remaining significantly reduced ($p<0.01$) over 60 minutes. Remarkably, filtration fraction (C_{inulin}/C_{PAH}) was unchanged from control (38 ± 2 %) throughout the reperfusion period.

Right kidney values for C_{PAH} did not differ from control (93.9±8.6 ml/min) for the first 20 minutes of reperfusion but tended to

decrease, by as much as 25% of control values after 40 minutes of reperfusion ($p<0.05$). C_{inulin} in the right kidney remained unchanged from control (34.7±1.9 ml/min) during ischemia and initial reperfusion. Values for C_{inulin} did tend to decrease in the final four sampling periods, by as much as 20% at 40 minutes of reperfusion ($p<0.05$), a phenomenon similar to that observed for C_{PAH}. Again, filtration fraction was not different from control at any time.

As seen in Table X, EB dye extravasation studies reveal that after 30 minutes of reperfusion, a significant decrease ($p<0.05$) from control values occurred in the left kidney inner medulla, from 763±51 to 586±60 μg EB/g dry tissue, and papilla, from 741±52 to 549±54 μg EB/g dry tissue. The regional distribution of EB dye in both the left kidney and right kidney after 60 minutes was dissimilar to that seen following 30 minutes of reperfusion. In the left kidney, comparison to control values revealed a significant increase in the outer medulla from 398±56 to 491±17 μg EB/g dry tissue ($p<0.01$) while increases seen after 30 min in the IM and PAP were no longer observed. In the contralateral kidney, outer medulla and inner medulla EB content was significantly increased ($p<0.01$, for both) from 409±29 to 524±34 and from 736±44 to 911±53 μg EB/g dry tissue. Right kidney cortex and papilla EB content was not different from control.

The ratios of wet/dry tissue were unchanged in either kidney after 30 minutes of reperfusion, as indicated in Table XI. Following 60 minutes of reperfusion, the ratio for each zone of the left kidney was significantly increased from 4.48±0.05 to 4.70±0.07 ($p<0.05$) for the CTX, from 5.10±0.19 to 6.00±0.10 ($p<0.01$) for the OM, from 7.13±0.37 to 8.26±0.17 ($p<0.05$) for the IM and from 6.35±0.03 to

7.66±0.29 ($p<0.05$) fo the PAP. In the right kidney, the small increase in water content observed in the OM and IM after 60 minutes of reperfusion, did not attain statistical significance when compared to control values.

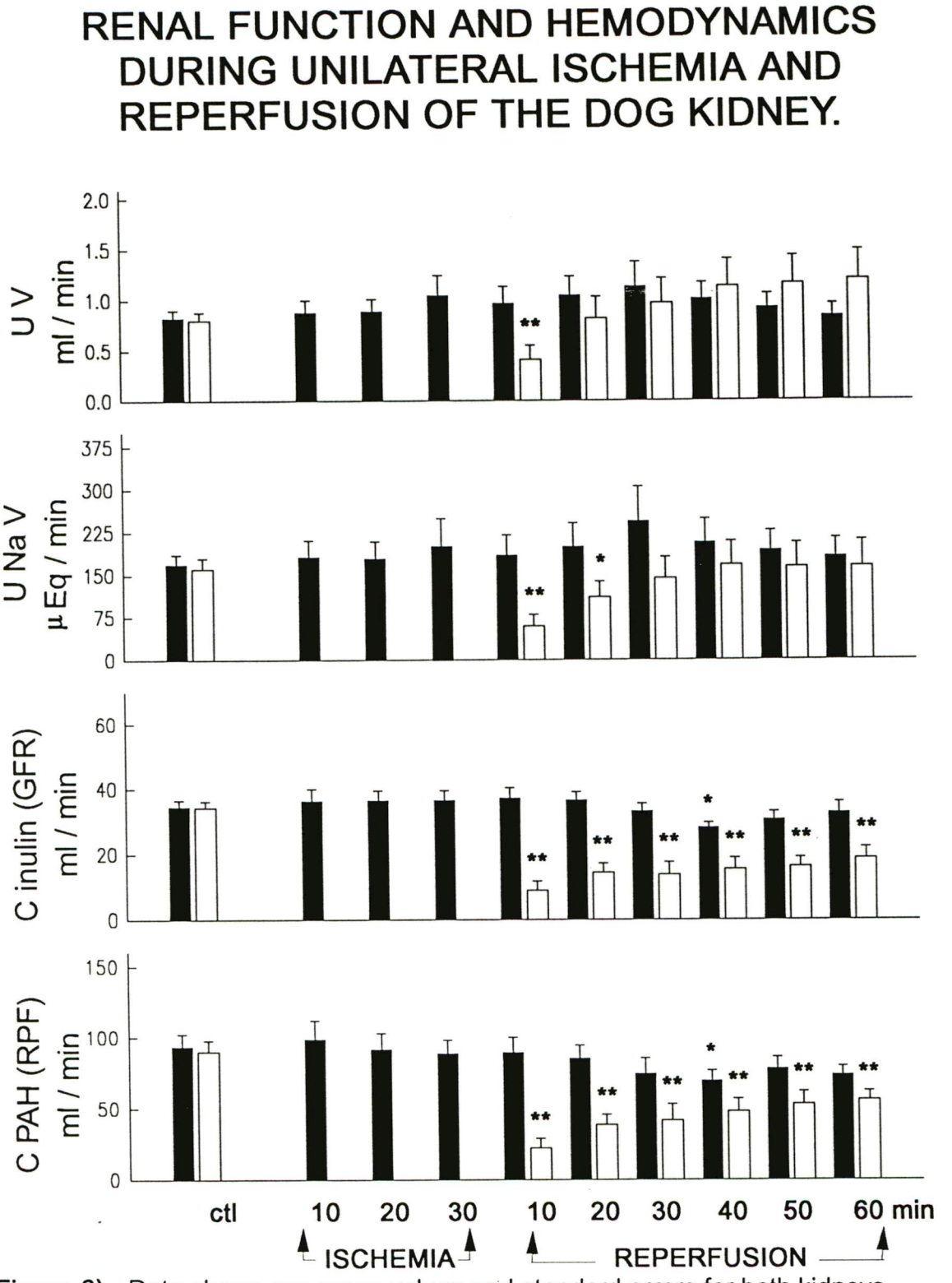

Figure 6) Data shown are mean values and standard errors for both kidneys before during and after left kidney ischemia. Results for the first 30 min reperfusion include all 13 dogs, those of the final 30 min are the results for 7 animals. Bars and significance symbols as in Figure 1 (see text for details).

REGIONAL EXTRAVASATION OF ALBUMIN BOUND EVANS BLUE DYE IN THE CANINE KIDNEY:

Changes over time during left kidney post-ischemia reperfusion.

LEFT KIDNEY	CONTROL N= 6	30 MIN REPERF. N= 6	60 MIN REPERF. N= 7
CORTEX	125±11	122±9	99±16
OUTER MEDULLA	398±56	386±31	491±17 **
INNER MEDULLA	763±51	586±60 *	782±38
PAPILLA	741±52	549±54 *	644±57

RIGHT KIDNEY	CONTROL N= 6	30 MIN REPERF. N= 6	60 MIN REPERF. N= 7
CORTEX	136±15	153±17	120±8
OUTER MEDULLA	409±29	464±54	524±34 **
INNER MEDULLA	736±44	828±67	911±53 **
PAPILLA	739±76	830±53	696±68

Table X) Values indicate µg of Evans Blue dye per g of dry tissue. Reperf. indicates post-ischemia reperfusion time. Significance symbols as in table IV (see text for details).

REGIONAL TISSUE WATER CONTENT

IN THE CANINE KIDNEY:

Changes over time during left kidney post-ischemia reperfusion.

LEFT KIDNEY	CONTROL N= 6	30 MIN REPERF. N= 6	60 MIN REPERF. N= 7
CORTEX	4.48±0.05	4.46±0.13	4.72±0.08 *
OUTER MEDULLA	5.10±0.19	5.30±0.20	6.00±0.10 **
INNER MEDULLA	7.13±0.37	7.09±0.26	8.26±0.17 *
PAPILLA	6.35±0.30	6.45±0.40	7.66±0.29 *

RIGHT KIDNEY	CONTROL N= 6	30 MIN REPERF. N= 6	60 MIN REPERF. N= 7
CORTEX	4.37±0.10	4.22±0.10	4.38±0.08
OUTER MEDULLA	5.19±0.15	4.88±0.23	5.37±0.14
INNER MEDULLA	6.80±0.42	6.67±0.35	7.27±0.26
PAPILLA	6.05±0.52	6.19±0.31	6.23±0.30

Table XI) Values indicate the ratio of wet over dry tissue weight. Reperf. indicates post-ischemia reperfusion time. Significance symbols as in Table IV (see text for details).

DISCUSSION

4.1 Functional effect of bradykinin during water diuresis and urea load.

As briefly mentioned in the introduction, the results obtained in previous studies on the renal functional effects of BK and selective receptor antagonists suggested altered function of the tubule segments beyond the thin ascending limb of Henle. Furthermore, we demonstrated that diuresis resulted from the action on B_2 receptors and that natriuretic effects resulted from action on B_1 receptors. The thick ascending limb of Henle has a high capacity for sodium chloride reabsorption (BANKIR and DeROUFFIGNAC, 1985; BANKIR *et al.*, 1987; JAMISON, 1973; MOLONY *et al.*, 1987) and BK mediated inhibition of solute reabsorption in this segment would result in enhanced natriuresis. Bradykinin and ADH receptors are not located on this segment in the dog, but active transport there is down regulated by the action of PGE_2 released from surrounding interstitial cells in response to bradykinin (FLAMENBAUM *et al.*, 1979; MOLONY *et al.*, 1987; NASJLETTI and MALIK, 1981). Action in the distal tubule and cortical collecting duct is possible since all the elements of the kallikrein kinin system are found there and that water and sodium may be reabsorbed independently or together under the influence of mediators such as ADH, PGE_2 and aldosterone. The selective reabsorption of sodium is known to occur under the influence of ADH in the outer medullary collecting duct while an effect of this hormone is known to mediate water and urea transport (via independent transport

mechanisms) in the papillary end of this segment (WALL *et al.*, 1992). Bradykinin receptors are known to be located on distal and collecting tubule segments (GUDER and HALLBACH, 1988). Furthermore, bradykinin has been shown to block ADH mediated water permeability in the collecting duct in vitro by an action on protein kinase C and PGE_2 release.

As we noted earlier, intrarenal blood flow redistribution has been suggested by many as a potential mechanism of BK action. Definitive answers as to what redistribution, if any, occurs are lacking due to the difficulties in accurately assaying regional blood flow in the kidney. However, it seems highly questionable that an increase in medullary plasma flow, resulting in the washout of the papillary osmotic gradient, is responsible for the diuretic effect of BK. The changes observed in the present and previous (LORTIE and PLANTE, 1990; LORTIE *et al.*, 1992) experiments occur within seconds and are consistently reversible within minutes, two findings incompatible with a medullary washout effect. It should be noted that the spatial arrangement of vasa recta tended to "insulate" the medulla from rapid fluctuations of its osmotic gradient in various species (BANKIR and DeROUFFIGNAC, 1985).The increase in blood flow measured using the transit time of markers does not differ between actual increased blood flow to the papilla and heightened flow due to free water flux into the ascending vasa recta.

Studies in water diuresis dogs where designed to demonstrate that bradykinin did not act by simply preventing ADH mediated events as was shown in vitro. The fact that we observed significant diuretic and natriuretic responses, without changes in global renal

hemodynamics, indicates that this is not the case. The effects seen here are dissimilar to those seen in normal animals since urine osmolarity was unchanged by bradykinin but was seen to decrease in normal animals. This suggests that bradykinin can not only regulate the independent reabsorption of free water and of sodium but also can regulate the bulk flow of water and sodium, as occurs in the connecting and cortical collecting segments.

Urea transporters and water channels are co-located on papillary collecting duct epithelium and are both under the regulation of ADH (KNEPPER and BURG, 1983; NADLER, 1990; NASJLETTI and MALIK, 1981). In untreated animals bradykinin caused an increase in free water clearance that could be explained in part by an action on the papillary collecting duct. Surprisingly, in spite of the increased urine flow, no change in the excretion rate of urea was observed. This implies that there is avid reabsorption of urea that only allows a small quantity to escape or that a constant secretion of urea ensures a steady rate of excretion, evidence for the latter in mammalian collecting ducts is fragmentary at best but is teleologically appealing since such a mechanism is known to exist in reptilian and avian kidneys.

The excretion of urea was unchanged by water diuresis or bradykinin in these experiments when compared to untreated animals. That bradykinin has no effect on ADH mediated urea transport under these conditions was not unexpected since levels of the latter hormone should be minimal, but that water diuresis itself didn't have a significant effect was somewhat disconcerting. This implies that ADH does not play an important role in the regulation of urea transport in

dogs or that the small differences in excretion rates we do see (1.5 mg/min) are physiologically important. In fact the difference observed amounts to a daily excess in urea excretion of a bit more than 2 grams and would incur an additional loss of approximately 350 ml/day of free water or 1700 ml of urine at 120 mOsm/l.

We conducted a similar series of experiments in urea loaded animals in an attempt to maximize urea transporter activity and because under these conditions antidiuretic mechanisms are expected to be fully activated. The infusion of urea caused a significant increase in diuresis, natriuresis and urea excretion as expected. The effect of bradykinin on diuresis and natriuresis was similar to that seen in water diuresis and untreated dogs. It is notable that the effects of bradykinin were not reversed rapidly after terminating the infusion, as was seen in every other condition studied. The author knows of no effect that urea might have on bradykinin degradation, receptor/hormone interaction or signal transduction that might explain this phenomenon.

As in untreated and water diuretic conditions, bradykinin had no effect on the excretion rate of urea. It is not clear exactly what effect urea loading might have on transporter activity in vivo since these have mostly been studied in vitro (BEYER and GELARDEN, 1988). The situation is paradoxical since the urea load must be excreted but the water loss incurred would surely signal the activation of antidiuretic mechanisms including urea accumulation in the papilla. In spite of the increase in urine flow the excretion rate of urea remained extremely constant and suggests that urea is transported into the collecting duct at a constant rate that is unaffected by bradykinin. As in untreated animals, bradykinin caused a decrease in urine osmolarity yet a net

increase in sodium and urea excretion occured. This implies a reduction in free water reabsorption as occurs in the collecting duct under the influence of ADH. The accompanying increase in sodium excretion may be in part due to natriuretic effects of bradykinin on other segments of the nephron as discussed for untreated animals. Finally, we can only speculate as to why urea loading caused a significant osmotic gap to occur and why it was eliminated by BK infusion. In fact it appears that a urea load obligates the loss of other osmolytes whose excretion arrear regulated by BK. This may reflect an adaptation that prevents undue loss of sodium in conditions of obligatory urea loss.

To summarize, previous studies lead us to conclude that bradykinin infused in the renal artery could independently increase diuresis and natriuresis via an action on different receptor subtypes (LORTIE *et al.,* 1992). It was proposed that the effects we observed could best be explained by action on sections of the nephron beyond the thin ascending limb since diuresis and natriuresis could be dissociated. We have just shown that bradykinin could induce a diuretic and natriuretic effect by means other than ADH antagonism since it did so during water diuresis. This and the fact that urine osmolarity was unchanged, suggested an effect on the connecting or cortical collecting duct. During osmotic diuresis induced by the infusion of urea, bradykinin was again able to cause a diuresis and natriuresis that could best be explained as the inhibition of ADH mediated free water reabsorption in the collecting duct. We also noted that urea loading caused an osmotic gap to occur that was eliminated by the infusion of BK. Finally, urea transport appears unaffected by

bradykinin and is excreted at an extremely constant rate over varying urine flow.

4.2 Albumin-EB binding under different pH or osmolarity

Spectrophotometer analysis of the maximal absorbance frequency of various EB solutions confirmed studies by others that EB binds tightly to albumin. We sought to determine what effect if any a change in pH or an increasing concentration of NaCl and urea might have on the binding of EB and albumin, since such conditions could be encountered in the renal medulla. In fact, urinary pH may reach values in the range of 4.5-4.7 whereas urine osmolarity can exceed 2000 mOsm in conditions of dehydration. It should be noted that the concentrations of EB and albumin used here are the same as those reported by RAWSON (1942), this to ensure that the binding capacity of albumin is not saturated as it has been shown that one albumin molecule can bind as much as 70 EB molecules (LeVEEN and FISHMAN, 1947). The concentrations of EB and albumin we use in these experiments, and in our *in vivo* studies, aim to achieve a 5 fold ratio of EB to albumin concentration. The negative results obtained from these experiments put to rest any doubts that the particular physical environment found in the renal medulla played a role in the very high concentrations of EB found there.

4.3 Systemic and renal vascular volumes (efficiency of renal vascular draining).

These studies were deemed necessary so that the relative contribution of vascular EB remaining in tissue samples could be assessed. Flushing of the renal microcirculation had been envisaged but the regional heterogeneity of the renal microcirculation and the potential risk of artifacts incited us to develop the simple draining technique described by Soltkoff and Lillienfield (1967). In fact, this group compared free draining to flushing and failed to demonstrate any appreciable advantage to flushing and actually appeared to wash out interstitial albumin. If tissue EB content is to be used in the assessment of albumin extravasation from the capillary compartment to the interstitial space, it is critical that the vascular content of EB marked albumin be insignificant. We demonstrated qualitatively and quantitatively the efficiency of rapidly removing and dissecting renal tissue to allow the free drainage of blood. The concomitant assessment of systemic vascular volume allows for the determination of renal vascular EB content.

The estimation of blood volume by the distribution volume of ^{51}Cr tagged RBC has been validated by others (BAUER *et al.*, 1975; ROTHE *et al.*, 1979) and the values we obtain are similar to those found in the literature. The average value is just over 100 ml/kg or 10% total body weight which is in agreement with the generally accepted approximation of plasma volume at 5% total body weight if one considers a blood hematocrit of near 50%. We were pleasantly

surprised at the small differences in blood volume measurements between animals, since mongrel dogs were used. This is particularly important since in all our different protocols the drugs and anesthetics administered are dosed according to body weight in an effort to achieve similar plasma concentrations. The systemic blood volume measured by us (108 ml/kg) reproduces values obtained by others (65-85 ml/kg and 98 ml/kg), BEYER and GELARDEN, (1988) and ROTHE et al. (1979), respectively, in similar sized dogs using the same technique.

The effectiveness of the renal vascular drainage in the present studies was nothing short of spectacular. By simply allowing free drainage and rapidly dissecting the kidney we were able to reduce remnant vascular volume in the kidney to less than 10%. We can use the net values for systemic volumes to estimate plasma EB concentration. Similarly, total and remnant renal vascular volumes are used to estimate renal vascular EB content. A 0.225 g/kg infusion of EB results in a whole blood concentration of less than 2.2 mg/ml. At this concentration, the intra-vascular EB contained in the remnant vascular volume would be less than 340 μg EB for the whole kidney (a whole kidney in these animals weighs between 60 and 85 g). This clearly obviates the negligible contribution by remnant vascular albumin since this amount of albumin can be recovered in 2 or 3 g of cortex tissue and even smaller samples from deeper zones.

We felt justified in pooling tissue samples from clamped and drained kidneys since the hematocrit, vascular volume and whole blood radioactivity showed little variation between animals. This allowed for less error due to nonspecific background noise (<45 CPM,

on both days of testing) while reading small samples. Unfortunately, since there are as of yet no precise figure reporting differences in the hematocrit values of renal zones in the dog, we are unable to estimate regional vascular volumes. However, the degree of vascular drainage can still be evaluated using the differences in radioactivity of tissue samples, when expressed in terms of their dry weight to account for difference in vascular volume weight. There is a small inherent error in this due to the dry weight of the intravascular fluid in the clamped kidney samples. The values so obtained reveal that drainage was more effective in the cortex that the medulla. The reason for this is not presently clear but was also observed by others (SOLTKOFF and LILLIENFIELD, 1967). With regards to the aforementioned study, it is clear that our methods of draining are more effective. Whereas their tissue samples retained 29, 32 and 61% of the original red blood cell volume in the cortex outer medulla and papilla (read inner medulla with papilla) we were able to achieve respective values of 6.5, 19 and 20.4%. Furthermore, in other experiments where they perfused kidneys with variable amounts of Ringer solution, flushing improved RBC removal by less than 50% in any of the zones and still did not achieve the degree of drainage we showed. It is most likely that the time required to start perfusion allowed for vascular congestion in their model and that the dissection we performed facilitated drainage in our tissues. A final point to note is the effect their flushing procedure had on extravascular albumin pool size. This was reduced by over 50% in all zones, particularly in the regions were vascular drainage was most effective, indicating that the flushing itself altered tissue albumin composition.

To summarize, we have shown that rapid excision, simple drainage and dissection effectively eliminates any potential contamination of intravascular EB marked albumin. Furthermore we show that vascular volume can be estimated accurately according to body weight since little variation in this parameter was seen between mongrel dogs.

4.4.1 Renal albumin extravasation studies in control dogs

The findings obtained with the EB technique raise a number of interesting points. First of all, the high reproducibility of this method is worthy of mention. The standard errors for EB content values and wet/dry tissue ratios are virtually all within 10% of the mean and as low as 2% for the cortex region. Note that EB content values in the present study are not necessarily indicative of the absolute size of an interstitial albumin pool, since equilibration is not complete in one minute (LASSEN *et al.,* 1958). Rather, since we have shown that intravascular albumin contributes little to tissue content in this model, regional differences in EB content must be due to different flow rates (albumin delivery), hydrostatic pressure and/or capillary permeability.

One of the most striking aspect of these results is the distinct heterogeneity in the distribution of EB in the different zones of the kidney. In no other organ or tissue studied so far have we observed the high concentration of EB seen in the IM and PAP, even after a prolonged circulation time. As noted previously, the rapid turnover of an extravascular albumin pool has been demonstrated by others in the renal medulla (GOLDBERG and LILLIENFIELD, 1963; KRIZ, 1982;

REGOLI *et al.,* 1990; WILDE and VORBERGER, 1967). The role of this pool in renal function has remained largely unexplained, perhaps due to difficulties in integrating it to urine concentration models which require the generation of an oncotic force gradient towards ascending vasa rectae.

It is not surprising to find that EB content in the CTX is the lowest in the kidney, considering that the development of high oncotic forces in peritubular capillaries is a key factor to passive proximal reabsorption mechanisms (BRENNER *et al.,* 1969). The low permeability to albumin of glomerular capillaries under normal conditions is partly due to the presence of negatively charged glycoproteins attatched to endothelial cells there. It may be that similar mechanisms exists in the peritubular microcirculation. Furthermore, since the CTX is the only region with lymphatic drainage in dogs (ALBERTINE and O'MORCHOE, 1979; LEMLEY and KRIZ, 1991) net increases in interstitial albumin and water are likely to dissipate. This does not preclude a possible increase in the rate of albumin and plasma extravasation in the CTX but would limit net accumulation in the interstitial compartment.

A greater accumulation of EB in the OM zone than in the CTX is not unexpected considering the dense capillary beds found there (KRIZ, 1982; PALLONE *et al.,* 1990). Also, this region is highly perfused with the ascending vasa recta of the IM and PAP which are fenestrated and permeable to macromolecules (KRIZ, 1982; PALLONE *et al.,* 1990; PALLONE, 1992; SHIMAMURA and MORRISON, 1973). The particularly high concentration of EB recorded in the IM and PAP reflects a very high rate of extravasation

there and perhaps a preferential passage of albumin from descending to ascending vasa recta via the interstitium.

The regional heterogeneity in the water content of the different zones reflects histological differences in cell types and interstitial matrix composition. In the renal CTX, the peritubular, periarterial and lymphatic spaces are interconnected and allow the free flow of fluid and macromolecules (ALBERTINE and O'MORCHOE, 1979). The very small variations in tissue water content here are likely due to a facilitated drainage of these compartments. In the canine kidney, the outer stripe of the OM is virtually nonexistent (BULGER *et al.*, 1979) while the interstitial volume of the interbundle region in the inner stripe is comparable to that of the CTX (LEMLEY and KRIZ, 1991). Nevertheless, the water content per gram of dry tissue in this region is consistently greater than in the CTX. This difference may be indicative of an important interstitial volume within the bundles themselves. The IM and PAP have the highest water content of the renal zones. It should also be recalled that all water reabsorbed from inner medullary collecting ducts passes through the interstitium of this tissue before returning to the systemic circulation via ascending vasa retae.

4.4.2 Renal function and albumin extravasation studies in control, water diuresis and hypertonic saline loading in dogs

The different functional responses we have documented under extreme differences in salt and water balances obviate the kidneys ability to dissociate salt and water excretion in an effort to maintain

body fluid homeostasis. These results reproduce those of many other studies (CHOU *et al.*, 1990; KNEPPER and BURG, 1983; PALLONE *et al.*, 1990; NADLER, 1990) and can be explained as the ability of the mammalian kidney to selectively reduce its reabsorption of either salt or water. In the examples at hand we note no changes in whole kidney RPF or GFR. This attests to the fine autoregulation of glomerular filtration in spite of changes in plasma levels of various vasoactive hormones that our experimental conditions would impose (vasopressin, atrial natriuretic peptide, etc). As noted earlier, these techniques cannot give insight into regional changes in vascular hemodynamic parameters. However, the concomitant measurement of albumin extravasation and of tissue water content in different regions of the kidney provides new information that locates intrarenal sites affected by these conditions. Also, these experiment may provide further insight into the physical parameters implicated in the extravasation of albumin in the renal microcirculation.

Some have proposed that the decrease in papillary flow following a salt load or ADH infusion results from a smaller volume of free water reabsorbed (and abstraction by ascending vasa rectae) from the papillary collecting ducts and an increase in reabsorption at the inner and outer medullary collecting ducts (see PALLONE *et al.*, 1990). The result of this would be to prevent a washout of the papilla by reducing the flow in the ascending vasa rectae there. These same arguments may be applied to explain data showing an increase in papillary blood flow during water diuresis. Here again, it is clear that flow rates are increased and a contributing factor to this may be that relatively more free water is reabsorbed at the papillary tip of the collecting duct under

these conditions while virtually none is reabsorbed in the OMCD. This was shown by Jamison et al. (1985) and was interpreted as a mechanism that would maintain a high osmotic gradient in the papilla during avid free water reabsorption under the effects of ADH and promote washout during water diuresis.

We observed in the IM and OM of salt loaded animals, an increase from control values in tissue EB and water content. However, only water content and not EB was seen to increase in the PAP in these animals. We also measured significant increases, compared to those of normal animals, in water and EB content of the OM, IM and PAP regions in the kidneys of animals undergoing water diuresis, while the CTX appears unaffected. These acute (<2.5 hours) changes we observe must be explained by increases in one or a combination of the following; blood flow (albumin delivery), hydrostatic pressure, vascular permeability and fluid reabsorption from the collecting duct (regulation of fluid reabsorption in the loop of Henle is unknown).

At first glance it appears difficult to reconcile these data with the fact that the medullary blood flow, and thus the delivery of marked albumin, is increased in one case and decreased in the other. In fact this strongly suggests that flow cannot by itself account for the extravasation of albumin in this region, the other factors must be considered as important. What is not clear is whether increased flow in the medulla could be expected to wash out not only urea and sodium but also the albumin pool in the medulla. This might in fact cause a decrease in maximal values obtainable in this region if the large pool of albumin in the medulla was generated by countercurrent multiplication, as proposed by Lassen (1958). This effect is minimized

in our conditions but could be resolved by allowing prolonged equilibration of marked albumin in water diuretic animals so that EB may be used to quantify interstitial albumin under different conditions.

Evidence that change in pressure alone can alter the endothelial permeability coefficient in itself is contradictory (eg. EHRRHART and HOFMAN, 1992; WILLIAMS and HUXLEY, 1993), however, extravasation of small solutes and macromolecules in plasma is subject to hydrostatic pressure gradients because of solvent drag (WILLIAMS and HUXLEY, 1993; also see MITCHEL, 1992). Interstitial pressure in the medulla of the kidney can be expected to change rapidly in response to hydrostatic pressure in vessels because the organ is surrounded by a strong, poorly compliant capsule membrane (KARAIBI and KNOX, 1989) and that this zone lacks lymphatic drainage (BULGER *et al.*, 1979). Thus changes in vascular hydrostatic pressure in the deep zones of the medulla would be felt by all structures found there and could alter their function. Relief of this pressure may be provided by a route of low resistance, the highly permeable ascending vasa rectae. Pallone (1992) has recently shown, in furosemide treated rats, that the diffusive efflux coefficient of both ascending and descending vasa recta was negligible but that convective transport was demonstrable in both and far greater in the former vessel. Put another way, simple diffusion is far less important than solvent drag and especially so for the ascending vasa rectae. This implies that vascular pressure could alter plasma ,and thus albumin, extravasation in the descending vasa recta and that interstitial pressure drives passage of fluid and albumin into the ascending vasa rectae. Recent unpublished data from Pallone

(personal communication) shows that descending vasa rectae tone can increase in response to angiotensin or norepinephrine and dilate in response to PGE_2. Furthermore it appears that it is the small vessels on the periphery of vascular bundles, surrounding those that perfuse the tip of the papilla, which show the greatest response. Because of its strategic location, post efferent arteriole vasoactivity could allow for intramedullary blood flow and pressure regulation without necessarily affecting glomerular hemodynamics.

As we have just noted, plasma sieving (solvent drag) appears to be the dominant factor mediating albumin extravasation. Permeability changes alone could certainly increase the rate of extravasation of marked albumin. Simple diffusion of marked albumin would also increase and might become a significant factor in situations of low vascular hydrostatic pressure (this would require that permeability increase independently of vascular tone). However, intravascular albumin concentration must be greater than that in the interstitium for this mechanism alone to cause a net increase in tissue albumin and consequently fluid volume. Many people have measured medullary plasma albumin concentrations and report high concentration at the papilla (see Pallone *et al.,* 1990). What remains elusive is the relative interstitial albumin concentration to determine the existence of an albumin concentration gradient.

Important information can be gleaned from these experiments with respect to the tissue water content which may be of vascular or tubular origin. In our studies, increases in albumin extravasation in the experimental groups are higher than control but not different from each other, however, the tissue water content values of water diuretic

dogs are significantly ($p<0.01$) greater than those of salt loaded animals. Our current understanding of renal function dictates that inner and outer medullary collecting duct permeability to water is minimized during water diuresis and maximized during salt load. Free water, formed in the thick ascending limb, is either excreted or passes, via the interstitium, to the ascending vasa recta and return to the general circulation. Interestingly no change is seen in the tissue EB content of the PAP of salt loaded animals and the change in tissue water content there is less dramatic than in IM. This may be related to the aforementioned mechanisms that minimize washout of the papillary tip (JAMISON *et al.,* 1985) by reducing blood flow and free water reabsorption, thereby enabling sustained urine concentrating power. Finally, one still has to invoke one or more of the factors described above (flow, pressure and permeability) to account for increased albumin extravasation.

To summarize, increases in albumin extravasation and water content were noted in the deep zones of the kidney in salt loaded and water diuretic dogs when compared to untreated animals. We have argued that plasma flow rates alone can not account for these results and that hydrostatic pressure, intramedullary blood flow redistribution and endothelium permeability are important factors in the phenomenon we observe. Furthermore, increased water content of these same zones in both experimental conditions may in part originate in the collecting duct and explain tissue water increases that are independent of albumin extravasation.

4.5 Albumin extravasation studies following the intrarenal infusion of bradykinin alone and concurrently with selective receptor antagonists

Altered flow, vascular hydrostatic pressure and the microvascular permeability to albumin are the factors that determine the rate of albumin extravasation under acute conditions. In animals receiving BK alone, increased RPF was not sustained and GFR did not change, furthermore BK is known to reduce vascular resistance (REGOLI and BARABE, 1980), thus we can suppose that hydrostatic pressure in the renal vasculature is not increased. The fact that we see no change in the tissue water content of left kidney CTX in this group, is not incompatible with increased plasma extravasation, since lymphatic drainage could prevent a net increase in interstitial fluid (BULGER *et al.*, 1979; LEMLEY and KRIZ, 1991). However, in the absence of an increase in hydrostatic pressure, a change in vascular permeability must occur. An increase in EB tissue content does not necessarily mean an increase in total interstitial albumin content but could be due to an increased turnover rate of the perivascular albumin pool.

Previous studies of ours indicate that either selective B_1 or B_2 receptor antagonists can inhibit the RPF increase caused by BK (LORTIE and PLANTE, 1990; LORTIE *et al.*, 1992), while either the diuresis or the natriuresis resulting from BK infusion can persist depending on the antagonist used. This evidence belittled the role of an increase in RPF in the natriuretic and diuretic effects of BK. However, redistribution of blood flow to the medulla could not be discounted since PAH clearance techniques might not be sufficiently

sensitive to identify significant changes in the small fraction of blood flow to this region. However, if BK rapidly changed medullary blood flow, one could expect that altered hemodynamics there would have an effect on EB accumulation and tissue water content, as observed in osmotic diuresis and water diuresis models.

The turnover of the albumin pool in the IM is not complete after one minute of perfusion since others have shown that albumin accumulation in the medulla continues even after 3 minutes of perfusion (LASSEN *et al.*, 1958). Therefore, an increase in EB content of the IM and PAP could be detected in our model. The lack of changes in both EB and water content does not favor altered medullary hemodynamics as the major mode of action for the natriuresis and diuresis that results from intrarenally infused BK.

Consideration of the physiological and pathophysiological implications of increased capillary permeability to albumin in the CTX are purely speculative at this point. However, such an effect would surely alter tubular and vascular exchanges normally driven by oncotic pressure gradients. Furthermore, there is no reason to believe that albumin is exclusively affected by these permeability changes. The perivascular, interstitial and mesangial deposition of other circulating macromolecules may also be altered. Finally, the fact that increases in both RPF and EB extravasation are inhibited by either antagonist is suggestive of a common mechanism.

4.6 Renal function and albumin extravasation profiles during equipotent doses of Captopril and Perindopril

In these experiments we chose to study subpressor doses of ACE inibitors since they are commonly used therepeutic agents that are known to reduce the degradation of BK. Infused via the renal artery avoided the activation of neurogenic and hormonal compensatory mechanisms acting on the kidney and allows for more clear observation of direct effects by the infused substances. We were unable to demonstrate any acute effects on RPF, GFR, $U_{Na}V$ or UV with the small doses of Captopril or Perindopril we infused.

In these two groups of animals, after thirty minutes of drug infusion, the renal tissue albumin extravasation and water content values were significanty different from control values and from each other. The profile observed in the group receiving Captopril was unlike any other we have seen to date. There was a significant unilateral decrease in albumin bound EB exclusivelly in the left kidney PAP and no other changes in any of the zones studied.

It is possible that the localized change we observe in the PAP could arise from a reduction in hydrostatic pressure exclusively in the vessels of that zone. The blood flow perfusing the PAP is provided exclusively from descending vasa rectae within the core of medullary vascular bundles (KRIZ, 1982). This subpopulation of vessels might preferentially vasodilate in response to Captopril, however, no receptors to angiotensin II have been reported in the vessels of the PAP and we saw no changes there following the infusion of BK

(although ACE was uninhibited in those experiments). A reduction in ascending vasa rectae tone, such that post capillary resistance is reduced, is improbable since it is hard to imagine any resistance by these vessels could be developed in the first place considering their extensive fenestrations. Also, there would have to be selective action by a hormone affected by ACE exclusively on the ascending vasa recta in the PAP or similar changes would be seen in the IM. Finally there is no change observed in tissue water content, a reduction might be expected if vascular hydrostatic pressure decreased. We know of no evidence that can support the idea that vascular hydrostatic pressure or permeability changes are responsible for the effects observed.

Another important factor to consider is blood flow, one explanation could be that a local reduction in the production of angiotensin II or a local increase in kinins preferentially dilates the vasa rectae perfusing the inner and outer medulla and thereby shunting blood destined for the PAP. However, most evidence indicates that Captopril causes an increase in papillary blood flow (HANSEL *et al.*, 1988; ROMAN *et al.*, 1988) that may be prostaglandin mediated (MATTSON and ROMAN, 1991; CUPPLES *et al.*, 1988) and deplete albumin concentration there by a washout effect. This could be easily verified by allowing a longer perfusion time. Without further experimentaion it is clearly impossible to formulate a tenable hypothesis as to how Captopril causes a reduction in albumin bound EB in the PAP.

A completely different profile was obtained during albumin extravasation studies when Perindopril was infused at a dose

equipotent to that of Captopril. In this case, although no changes in systemic blood pressure were noted, identical effects were seen in both kidneys. In both the IM and OM there was a significant increase seen in the extravasation of albumin. Interestingly, it was only in the OM of each kidney that a significant increase in tissue water content was seen.

Experiments in rats and monkeys show a high concentration of angiotensin II receptors located in the inner stripe of the OM (MENDELSOHN *et al.,* 1987), more recently these have been specifically localized on type 1 interstitial cell (ZHUO *et al.,* 1992) (we noted earlier that the OM of the dog is comprised almost entirely of inner stripe). This location of receptors implies that there is either extravasation or local production of angiotensin II. Type 1 interstitial cells are known to produce vasoactive and permeabilizing prostaglandins in responce to bradykinin and angiotensin II (BROWN *et al.,* 1980; KURDORA et al 1979). Selective action at these sights coupled with the lack of effect in the PAP, such as we observed with Captopril, is most likely due to the particular pharmacokinetic characteristics of Perindopril. The fact that identical changes were seen in the contralateral kidney suggests that this subtance has a particular affinity for certain tissue types or isozymes of ACE. If effects on the contralateral kidney are due to recirculated Perindopril the effective dose for this effect must be orders of magnitude less that used in the present study.

It is unclear why tissue water content increased in the OM and not in the IM following Perindopril infusion. The vascular bed of the OM, which perfuses the thick ascending limb of Henle, is more elaborate

and carries more blood than that of the IM (KRIZ, 1982) yet there is consistently less albumin extravasation seen in this zone. The fact that we observe extravasation of both plasma and albumin in the OM and only albumin in the IM following Perindopril also suggests that the vessels of the OM have structural or functional characteristics which are different from those of the IM.

In summary, we have shown important diferences in the renal location of effects following equipotent doses of two different ACE inhibitors with respect to albumin and fluid extravasation. Furthermore, these were seen to occur at doses that did not alter systemic or renal artery blood pressure, renal hemodynamics and did not affect renal diuresis or natriuresis. The implications and repercussions of these results require further testing, it may be interesting to compare the urine concentrating abilities following treatment with these drugs since both preferentially affect the medullary zones but in a substantially different manner.

4.7 Changes in renal function and albumin extravasation after 30 or 60 minutes of post-ischemia reperfusion

The renal function data presented here shows clearly that dynamic changes in renal function occur in the initial phase of reperfusion following 30 min renal ischemia. UV and $U_{Na^+}V$ were fully restored in the LK within 30 min of reperfusion, while C_{PAH} and C_{inulin} remained significantly reduced (although filtration fraction was unaltered). This appears to indicate a dissociation of tubular function and renal hemodynamics involving a decrease in net sodium and

water reabsorption. However, the parallel reduction of C_{PAH} and C_{inulin} and maintenance of filtration fraction is indicative of intact autoregulatory mechanisms. Although these mechanisms can operate over a wide range of renal perfusion pressure and flow, it is unlikely that filtration fraction would be maintained during a 75% reduction in RPF. Furthermore, studies in dogs report that 90 min. renal ischemia resulted in an increased RPF upon reperfusion, measured by electromagnetic flowmetry, but a simultaneous decrease in C_{PAH} and C_{inulin}, suggesting a nonselective backleak of filtrate (RILEY *et al.*, 1975).

The decrease we observed in RK hemodynamics, and the declining trend in both UV and $U_{Na^+}V$, after 40 min of reperfusion is interesting and suggests an interaction between kidneys. An increase production of circulating vasoconstrictors such as ANG II by the LK could account for the decrease in RK hemodynamics. However, since systemic cardiovascular parameters were not altered, a more selective mediator (circulating or neurogenic) of renal hemodynamics would have to be considered.

The data derived from regional tissue EB and water content provides evidence of changes in intrarenal microcirculation which could either be the cause or consequence of the altered renal function observed during reperfusion. In the LK, a significant decrease in albumin bound EB in the inner medulla and papilla without any change in tissue water content was seen after 30 min of reperfusion. This could result from reduced perfusion in these zones and/or decreased extravasation of albumin.

After 60 min of reperfusion, the EB content of each zone in the LK is different from that observed at 30 min. The decrease in EB content in the left cortex could be the result of a reduction in renal perfusion (the onset of a "no-reflow" phenomenon) or due to a washout of interstitial albumin from this zone. In support of this latter concept, others have shown that the flow and albumin content in the renal lymphatics is augmented following I-R (HELLBERG *et al.*, 1990).

A significant increase in outer medulla EB content could be indicative of an increase in delivery and/or extravasation of albumin. No evidence is known to us that indicates a selective increase in outer medulla perfusion, on the contrary, hypoperfusion has consistently been reported. It has been proposed that local hemoconcentration could result in the sludging of erythrocytes, leukocytes and platelets in this zone (HELLBERG *et al.*, 1990; HELLBERG *et al.*, 1990; KARLBERG *et al.*, 1982). Increased vascular permeability to plasma albumin in the outer medulla could play an instrumental role in this phenomenon by reducing the oncotic pressure gradient towards the capillary lumen.

The return to control values of LK inner medulla and papilla EB content after 60 min of reperfusion indicates that the plasma flow and/or permeability characteristics of these zones is preferentially restored. This is in agreement with others who have shown an increase in papillary blood flow after ischemia and suggested that shunting of plasma normally destined for the outer medulla, because of erythrocyte congestion, may help to restore perfusion to the deep medullary region (YAGIL *et al.*, 1989). Furthermore, the deep medullary circulation may be resistant to congestion due to

hemoconcentration since this is thought to occur normally when blood flows through the high osmotic gradient there.

A general edema becomes apparent in the reperfused kidney. The fact that we observe an increase in cortex water content is surprising since it is subject to lymphatic drainage. The increased water content in this case can best be explained by an increase in cell volume, possibly as a result of impaired voloregulatory ion transport. In the other zones, increased interstitial volume could account for the increased tissue water content, but changes in intracellular volume cannot be excluded.

The significant increases in EB content found in the inner and outer medulla of the contralateral kidney after 60 min of reperfusion, without apparent change in tissue water content, is particularly interesting. An increase in medullary perfusion could be proposed, but the papilla does not seem to be affected. Accordingly, it would appear that the vascular permeability of the microcirculation in these zones is selectively affected. A renorenal reflex mechanism could be responsible and would easily be verified using a model of renal denervation. Another explanation for increased EB extravasation in the RK outer medulla derives from the reports cited in the introduction that suggested the potential for immunospecificity towards the renal microcirculation. Such a mechanism would necessitate the immunorecognition of cell surface markers unique to the tissue in affected renal zones.

To summarize, we have shown in this study that tubular dysfunction is immediately apparent in the first minutes of reperfusion. UV and $U_{Na^+}V$ are rapidly restored while the reduction in the C_{PAH}

and C_{inulin} persists. Since filtration fraction is maintained, in spite of a 75% decrease in C_{PAH}, these results could be explained by the idea that ischemic damage to the proximal tubule allows for nonselective backleak of these markers. We have also demonstrated a time course progression of intrarenal changes in EB bound albumin extravasation and tissue water content. Changes in deep medulla microcirculation are evident after 30 min of reperfusion but not at 60 min. This supports the view that perfusion, and perhaps permeability changes, in deep medullary zones is rapidly restored. We have shown that change in outer medulla and cortex EB content, as well as a general edema, becomes apparent only after 60 min of reperfusion. This suggests a selective action by or the local release of vasoactive or permeabilizing factors. This data suggests that the extravasation of albumin can contribute to local hemoconcentration in the outer medulla but not in the deeper zones where we normally find a very large extravascular albumin pool. Changes in contralateral renal function and EB content, without systemic cardiovascular changes, suggest the participation of mediators or a delayed renorenal reflex response acting selectively on the kidney.

CONCLUSIONS

5.1 Functional effects of bradykinin during water diuresis and urea load.

Both water diuresis and urea load cause a significant increase in diuresis and natriuresis with insignificant changes in renal hemodynamic parameters.

The dose of bradykinin infused into the left kidney (0.05 μg/kg/min) does not alter systemic blood pressure or contralateral kidney function in either urea load or water diuresis dogs.

The intrarenal infusion of a low dose of exogenous bradykinin under either condition causes a sustained unilateral increase in urine flow and sodium excretion. A lack of change in renal hemodynamic parameters implies a tubular effect of kinins.

Urea excretion is unchanged by BK infusion in both water diuresis and urea load dogs. The notable stability of this parameter in the face of changed urine flow and sodium excretion indicates a tight regulation of urea excretion that is not mediated by bradykinin.

Water diuresis causes a significant reduction in urine osmolarity that is unaffected by BK infusion. This and the fact that renal hemodynamics appear unchanged suggests that increased diuresis and natriuresis results from an effect on a distal tubular site, where both water and salt are reabsorbed together, such as the cortical collecting duct. Furthermore, these effects are not due to the inhibition of ADH mediated effects since water diuresis suppresses the release of this hormone.

Urea load causes a significant increase in urine osmolarity that is significantly reduced by the infusion of BK. This indicates an increase in free water reabsorption as occurs in the inner medullary collecting duct. Urea load is known to induce ADH secretion and therefore part of the effects observed are likely due to the inhibition by BK of its effects on free water reabsorption.

The apparent inhibition of ADH mediated water reabsorption but lack of effect on urea transport (also mediated by ADH) by BK is indicative of post receptor interaction and that urea and water transport are independently regulated.

5.2 Albumin-EB binding under different pH or osmolarity

Changes in pH and osmolarity have no effect on the binding of albumin and Evans Blue dye.

High concentrations of dye in the low pH and high osmolarity environment of the medulla are not due to dissociation from albumin.

5.3 Systemic and renal vascular volumes (efficiency of renal vascular draining)

The total renal vascular volume in the dog is approximately 0.1 % of the total systemic volume.

The remnant renal vascular volume after free drainage is less than 8% of the total renal vascular volume.

Drainage is less effective in the medulla than in the cortex but remnant vascular is nevertheless reduced to near 20%.

The quantity of marked albumin in the remnant vascular volume of renal tissue samples is negligible and cannot account for that measured in renal tissue samples. There is no doubt that the Evans Blue measured in these studies is virtually all extravascular.

5.4.1 Renal albumin extravasation studies in control dogs

The distribution of marked albumin in the kidney is heterogeneous and likely reflects capillary permeability differences since the relative values do not reflect blood flow or hydrostatic pressure differences

Regional differences occur in renal tissue water content, reflecting regional differences in cell types and interstitial volume.

The method used to measure albumin extravasation is highly reproducible with standard errors always less than 10%.

5.4.2 Renal function and albumin extravasation studies in control, water diuresis and hypertonic saline loading in dogs

Both hypertonic saline infusion and water diuresis cause a significant increase in diuresis and natriuresis. However urine flow rate is more important in water diuresis and sodium excretion is far greater in hypertonic saline infusion dogs.

Renal hemodynamic parameters are unaffected by these experimental conditions

Hypertonic saline infusion selectively increases the extravasation of albumin in the outer and inner medulla. Also, these conditions result in an increase in tissue water content in all three deep zones of the kidney.

Water diuresis impacts on the medullary zone. Albumin extravasation increases significantly in the outer and inner medulla as well as in the papilla. Tissue water content also increases in these three zone, achieving values that were significantly greater than those observed in hypertonic saline infusion.

The mechanisms involved in these changes likely include permeability and/or hydrostatic pressure changes since flow to the medulla is decreased in hypertonic saline infusion. Free water reabsorption may account for increased tissue water content in the latter condition.

5.5 Albumin extravasation studies following the intrarenal infusion of bradykinin alone and concurrently with selective receptor antagonists

Exogenous bradykinin infused in the renal artery selectively increases albumin extravasation in the renal cortex without altering tissue water content in this region.

The co-infusion of either a B_1 or a B_2 receptor antagonist with bradykinin completely inhibits the extravasation of albumin.

As with previous results showing similar effects on renal plasma flow, it appears that in the dog renal vasculature, bradykinin exerts its effects by acting on both receptor subtypes.

5.6 Renal function and albumin extravasation profiles durring equipotent doses of Captopril and Perindopril

The doses of ACE inhibitors we infused intrarenally have no effect on renal function.

Captopril significantly reduces the extravasation of albumin in the papilla. No other effects were noted.

An equipotent dose of Perindopril, 10 times less, causes a bilateral increase in albumin extravasation in the outer and inner medulla and an increase in tissue water content in the outer medulla.

Thus these two related pharmacological agents have different sites of renal action. Furtheremore, Perindopril was either recirculated to the contralateral kidney and is effective at very low doses or it regulates circulating and/or neurogenic factors selective for the medullary vasculature.

5.7 Changes in renal function and albumin extravasation after 30 or 60 minutes of post-ischemia reperfusion

In the first 10-20 minutes of post ischemic reperfusion, urine flow and sodium excretion return to baseline values but the clearance of inulin and PAH remains significantly depressed.

The extravasation of albumin in the inner medulla and papilla, diminished after 30 minutes reperfusion, returns to baseline values after 60 minutes. This supports the idea of a rapid restoration of blood flow to this region after ischemic injury.

After 60 minutes of reperfusion an increase in both albumin extravasation and tissue water content becomes evident in the outer medulla. The time course of these events indicates that reperfusion leads to the release of vasoactive mediators acting on the microcirculation in this region.

The fact that a similar increase in albumin extravasation is seen in the contralateral kidney suggests that the mechanisms involved include either renorenal reflexes or the selective action in this capillary bed of circulating factors.

Increased tissue water content, after 60 minutes of reperfusion, in the inner medulla and papilla of the left kidney without accompanying change in albumin extravasation likely results from cell swelling.

5.8 Final remarks

There is evidence to show that kinins play a role in renal physiology and pathophysiology. By deduction, using renal functional data under various conditions, and by direct measurement of albumin extravasation and tissue water content, we have succeeded in identifying some of the tubular and microvascular effects of bradykinin. We have seen that pharmacological manipulation, by introducing exogenous or increasing endogenous kinins, result in distinctly different effects. Furthermore, in a common type of renal injury, known to involve vasoactive mediators such as kinins, still another profile of microvascular perturbation is observed. These results are indicative of the multiple effects and complex interaction of hormone mediators acting on both vascular and tubular elements of the kidney.

The development of albumin extravasation studies in the kidney has proven to be a valuable tool allowing the rapid characterization of the renal microcirculation. This has helped to answer, and certainly raise, a number of questions with regards to the renal role of kinins and we hope that the results of the present studies will stimulate interest into their modes of action in the kidney. Finally, these results raise questions as to the mechanisms of renal function in general. We would like to reemphasize that the regional control of albumin extravasation may be of particular importance in the kidney and is deserving of further consideration.

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